An Electronic Nose to Classify Iberian Pig Fats with Different Fatty Acid Composition

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ABSTRACT: Fatty acid analysis is frequently performed in fat and other raw materials to classify them according to their fatty acid composition, but the need to carry out online determinations has generated a growing interest in more rapid options. This research was done to evaluate the ability of a polymer-sensor based electronic nose to classify Iberian pig fat samples with different fatty acid compositions. Significant correlations were found between individual fatty acids and sensor responses, proving that sensor response data were not fortuitously sorted. Significant correlations also appeared between some sensors and water activity, which was considered during the sample classification. Two supervised pattern recognition techniques were attempted to process the sensor responses: 85.5% of the samples were correctly classified by discriminant analysis, but the percentage increased to 97.8% using a one-hidden layer back-propagation artificial neural network. The electronic nose (specifically, sensor responses analyzed by a neural network) achieved success similar to that obtained using the more usual fatty acid analysis by gas chromatography.

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KEY WORDS: Electronic nose, fatty acids, Iberian pig, pattern recognition, polymer gas sensors.

Pig fat or lard is a common ingredient in the production of a large variety of foodstuffs. Its fatty acid composition influences not only its fluidity but also its oxidative stability and odor development. Furthermore, fatty acid composition of fat is currently used for classifying purposes by the Iberian pig industry (1,2), a sector of the Spanish meat industry that usually produces dry-cured products of high value. In spite of its widespread use, some characteristics of the analytical procedure (time-consuming, cumbersome, requirement of equipment and personnel) restrict its application online. Therefore, methods that allow a quicker classification are in great demand.

To date, procedures for controlling quality in raw material and processed foods by analyzing their odors (sensory and volatile compound analyses) have usually been unfeasible. In recent years, the appearance of the electronic nose has led to the possibility of monitoring odors in a rapid way, although some attention to the sensitivity of these instruments to humidity must be paid, especially sensors that work at low temperatures (3,4). The relationship between fatty acid composition and the volatile compound profile generated already has been established for dry-cured Iberian pig samples (5,6) and also has been used to demonstrate the feasibility of the electronic nose to distinguish oil samples that have undergone different oxidative conditions (7). Therefore, electronic noses could be used to classify fat samples with different fatty acid composition, since their degradation by oxidative reactions yields different volatile compounds that cause a characteristic response in gas sensors. Up to now, electronic nose performance has mainly been compared to odor sensory analysis or volatile compound analysis by gas chromatography–mass spectroscopy (8), which could be considered as directly related to odor but which cannot be applied online. That is, there has been no comparison to the usual techniques for quality control, which focus on characteristics such as fatty acid composition, without directly considering volatile compound composition.

The objective of our research was to study the ability of an electronic nose to distinguish samples with small differences in fatty acid composition, because these types of samples usually involve the most difficulty.

EXPERIMENTAL PROCEDURES

Samples. Samples of subcutaneous adipose tissue were dissected from 30 Iberian pigs immediately after slaughter. All the samples were collected on the same day, and were taken from three different anatomical locations ($n = 30 \times 3$): on gluteus medius muscle (group A), on semitendinosus and semimembranosus muscles (group DO), and next to the coccygeal vertebrae (group H). They were vacuum-packaged, frozen and kept at –80°C until needed for analysis. Before the analysis, the samples were minced and blended.

Measurements with the gas sensor instrument. An AromaScan A32/8S electronic nose (AromaScan plc, Crewe, Cheshire, United Kingdom) was used, which comprises a 32-element sensor array of conducting polymers. Samples (5 g) were placed in 60-mL odorless glass vials, sealed, and maintained at 30°C for 10 min prior to resealing them with the sampling lid. (This procedure allows us to carry out the first heating period for a sample at the same time as the headspace equilibration and the sensor measurement for the other sample.) Samples then were held for 5 min at 30°C to let the sample equilibrate with the headspace. A data acquisition cycle was carried out: 30 s to equilibrate the sensor responses at 10% relative humidity air (previously filtered through charcoal and molecular sieve); 130 s (from 30 to 160 s) for sensor

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measurement, using a filtered air flow at 10% relative humidity to carry the volatile compounds from the headspace to the sensor chamber; and 200 s to wash the sensors using vapor generated from a 2% butanol-in-water solution. Empty glass vials also were sampled after each cycle. Four replicates were analyzed of each sample, and special attention was paid to use a random sampling order.

Sensor response values were expressed as $R_s \times 1000/R_o$, where R_s was the sensor resistance for the sample and R_o was the sensor resistance for filtered air (filtered through charcoal and molecular sieve) at approximately 10% relative humidity. Sensor responses were studied as normalized data, which were averaged over a fixed time range.

Water activity measurement. Water activity (A_w) was determined with a GBS Scientific Instruments FA-st/1 system (Romans, France). Two replicates were analyzed of each sample.

Fatty acid analysis. Fatty acid composition was determined by gas chromatography of the fatty acid methyl esters synthesized by using methanolic hydrogen chloride, as described by Carrapiso *et al.* (9). Solution (0.1 µL) was injected into an HP 5890II gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a cold on-column injector, a flame-ionization detector, and a 30 m × 0.53 mm capillary column coated with FFAP-TPA stationary phase (1 µm thickness). The conditions were as follows: oven temperature 220°C isothermal for 30 min, injector and detector temperature 230°C, flow rate of the carrier gas (nitrogen) 2.6 mL/min. Two replicates were analyzed of each sample.

Data analysis. Statistical analyses were performed on the mean of all the replicates of each sample. One-way analysis of variance and the Tukey test were used to compare means for A_w and fatty acid values (10). A nonlinear mapping algorithm (the Sammon map method) that preserves as much as possible the internal data structure (11) was used to select points of analysis in the sensor responses. Pearson correlation (10) and factor analysis [using principal components analysis (PCA) as the method for factor extraction] (12) were applied to evaluate the relations among variables. Discriminant analysis (DA) by stepwise procedure was used to select the most useful variables for distinguishing among groups and to classify samples (12). A standard one-hidden layer back-propagation artificial neural network (ANN) also was used to sort the sensor responses; and the nodes in both the hidden and the output layers had sigmoidal transfer functions (13). The discriminant functions and the trained ANN were not validated against a separate data set.

Statistical analyses were performed by means of the AromaScan A32S version 1.21 (which included the Sammon map method) and the SPSS version 9. The neural network analysis was carried out using an evaluation tool (Neurosolution version 1.3).

RESULTS AND DISCUSSION

Fatty acid composition and A_w. Fatty acid analysis showed small but significant differences among the three groups of samples (Table 1), as expected (14). Significant differences also appeared in A_w (Table 1) between A and H (lower values) and DO (higher values), which must be considered during sensor data analysis to avoid humidity interference or a sample classification significantly influenced by A_w instead of being mainly conditioned by volatile compound profile. Even though water vapor is a characteristic volatile from some types of samples (15), that is not the case with fats. Differences in A_w in these samples are not related necessarily to fatty acid profile; hence, sensors highly correlated to A_w should be researched.

TABLE 1
Fatty Acid Composition and Water Activity Values of Subcutaneous Fat Samples[a]

Fatty acids	Fatty acid composition (g fatty acid/100 g total fatty acids)			*P* values
	A[b]	DO[c]	H[d]	
14:0	1.5 ± 0.1[b]	1.6 ± 0.1[a]	1.6 ± 0.1[a]	<0.001
16:0	22.1 ± 0.8[c]	22.9 ± 1.2[b]	24.8 ± 0.8[a]	<0.001
17:0	0.4 ± 0.1[a]	0.3 ± 0.1[a,b]	0.3 ± 0.0[b]	0.021
18:0	10.1 ± 0.7[b]	9.1 ± 0.8[c]	10.9 ± 1.0[a]	<0.001
20:0	0.2 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.0[c]	<0.001
16:1	2.3 ± 0.2[c]	2.8 ± 0.4[b]	3.0 ± 0.4[a]	<0.001
17:1	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.3 ± 0.1[b]	<0.001
18:1	52.2 ± 1.1[b]	53.0 ± 1.5[a]	49.1 ± 1.1[c]	<0.001
20:1	1.8 ± 0.2[a]	1.6 ± 0.2[b]	1.2 ± 0.1[c]	<0.001
18:2	8.7 ± 0.5[a]	7.8 ± 0.5[b]	8.1 ± 0.8[b]	<0.001
18:3	0.4 ± 0.1[b]	0.4 ± 0.1[b]	0.5 ± 0.2[a]	<0.001
	Water activity			
A_w	0.85 ± 0.06[b]	0.94 ± 0.04[a]	0.86 ± 0.07[b]	<0.001

[a]Data appear as mean values ± standard deviation. Means within a row followed by different roman superscript letters are significantly different ($P \leq 0.05$).
[b]Samples taken on gluteus medius muscle.
[c]Samples taken on semimembranosus and semitendinosus muscles.
[d]Samples taken next to the coccygeal vertebrae.

Selection of the point of analysis in sensor responses. In order to evaluate the sensor responses, initial (up to 40 s) and final (155 to 160 s) data were eliminated because of their instability. Sensor responses were divided into three data sets, which were analyzed using the Sammon map method. The most useful period was clearly found at 120–155 s. Even more discrimination of samples was achieved at 150–155 s. The classifying power at those periods was also checked by using DA, and the interval 150–155 was also the best. Therefore, this period was taken as the point of analysis.

The best results were obtained using no initial measuring intervals, likely because of the type of sensors used, which typically show a larger sensitivity in their response by increasing the time of interaction between gas sensors and volatile compounds (16) before the occurrence of sensor saturation. Rocha *et al.* (17), also using an AromaScan A32/8S electronic nose, reported inadequate final values owing to the saturation of the sensors with humidity. In our study, the humidity in the sensor array chamber also rose during the sampling process, but the result was the opposite: the most useful values were obtained when the humidity was higher, probably because the humidity content of the headspace was insufficient to interfere with the sensor response to volatile compounds.

Relation among variables. PCA was used to obtain a simplified view of the relations among quantitative variables (individual fatty acid values, sensor values taken at 150–155 s, and A_w values). Figure 1 shows that most of the sensor values are plotted in two groups, which means that most sensor data are redundant and correlated, whereas the seven nongrouped sensors have information related to A_w or some fatty acids.

As expected, most correlations among sensor data were significant (Pearson correlation), confirming that much of the information may be redundant, which is typical of sensors made of conducting polymers (4). Furthermore, highly significant correlations ($P < 0.001$) between five sensors (S9, S13, S20, S29, and S32) and A_w were found. A considerable influence of A_w on sample classification could be ruled out because of the low classifying power of these sensors, insufficient to be included on the discriminant functions during the later discriminant analysis (Table 2). In any case, these sensors were not used in the later data classification carried out with the neural network. Correlation was also found among fatty acid profiles and sensor values. The most significant correlations appeared among the A_w-uncorrelated sensors and the fatty acid which had significant differences among groups. Therefore, the relation between fatty acid composition and sensor responses is clear. In fact, fatty acids are involved in an important way in odor development by lipid oxidation, and, as was mentioned above, the feasibility of using the electronic nose for detecting odors produced by oxidation of corn oils has already been established (7). In our raw fat samples these reactions were limited from slaughter until storage at –80°C, but they could be significant during the stabilization at 30°C prior to the sensor measurement.

Classification of data. The classification of samples with different fatty acid compositions was performed by using a DA, and an ANN, given that they currently seem to be the most useful techniques for classifying samples using sensor data from electronic noses (16). Three variables were chosen by DA (Table 2), with the last variable providing only a small improvement in the model. Note that none of the sensors highly correlated with A_w was selected, as was mentioned above. The two discriminant functions were highly significant ($P < 0.001$) and displayed a canonical correlation of 0.772 and 0.628. Therefore, 75.5% of the variance in the dependent variable can be explained by these functions. Only 85.5% of the samples were correctly classified, which translates to poor classifying performance. The data from the DO group were difficult to sort; in fact, only 73.3% of the samples from this group were correctly classified, probably because of their higher A_w. In any case, the three groups representing different fatty acid compositions were quite separate in the space being examined (Fig. 2).

The electronic nose performance using ANN (98.9% of the samples were correctly classified) (Table 3) was better than that using DA (85.5%). This result is in accordance with different reports that have shown the usefulness of neural networks for the sensor data analysis, due not only to less strict

TABLE 2
Variable Selected By Stepwise Procedure in Discriminant Analysis[a]

Variable[b]	Step[c]	Wilks' lambda value[d]	*F*-value[e]	Significance[f]
S26	1	0.41	63.26	0.000
S17	2	0.27	40.14	0.000
S25	3	0.24	28.95	0.000

[a]Variables are entered sequentially according to the discriminatory power they add to the model.
[b]Variables entered in the model.
[c]Number of step where each variable was selected.
[d]Wilks' lambda value, i.e., the significance criterion to enter $P < 0.05$.
[e]Value associated with each variable.
[f]Statistical significance.

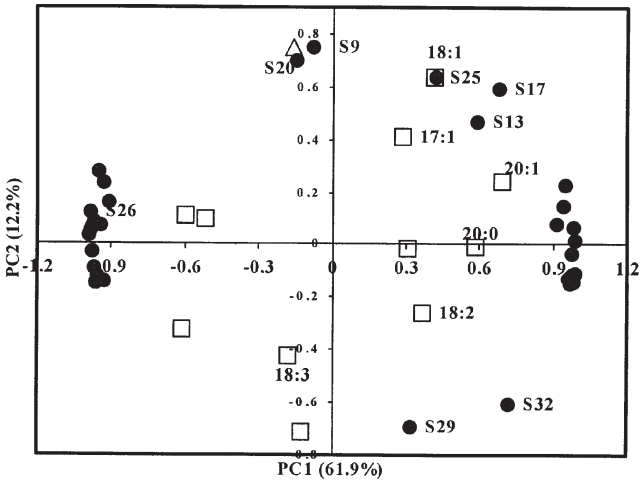

FIG. 1. Projection of the variables onto the space defined by the two first principal components (PC1/PC2). Variables: (□) individual fatty acids; (●) sensor values; (△) water activity. Variables of interest (individual fatty acids or sensor values included in discriminant functions, and sensor values correlated to water activity) are labeled.

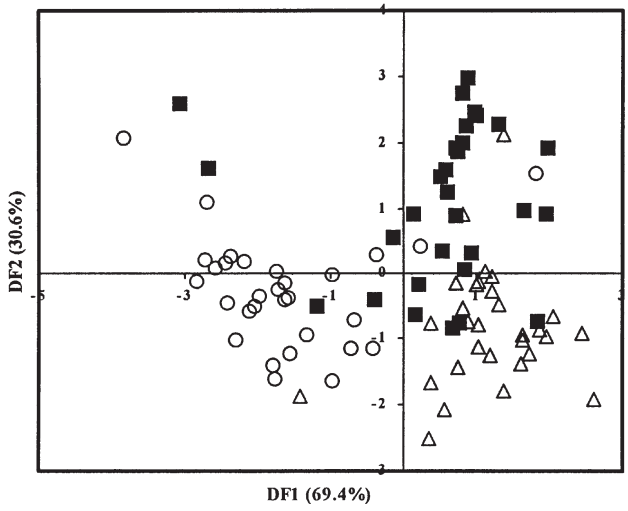

FIG. 2. Projection of the samples onto the space defined by the two discriminating functions (DF1/DF2). Each point represents the mean of four measurements from an individual sample. Sample groups: (○) group A (samples taken on gluteus medius muscle); (■) group DO (samples taken on semimembranosus and semitendinosus muscles); and (△) group H (samples taken next to the coccygeal vertebrae).

TABLE 3
Comparison of the Classifying Performance of Conventional Fatty Acid Analysis by Discriminant Analysis and Sensor Measurement By an Artificial Neural Network

	Samples correctly classified (%)[a]	
Group	Fatty acid composition	Sensor responses
A[b]	29 (96)	29 (96)
DO[c]	29 (96)	30 (100)
H[d]	30 (100)	30 (100)

[a]Values in parentheses are percent correctly classified.
[b]Samples taken on gluteus medius muscle.
[c]Samples taken on semimembranosus and semitendinosus muscles.
[d]Samples taken next to the coccygeal vertebrae.

requirements of the data but also to marked improvement in classifying tasks (4). Sensor measurement–neural network treatment achieved a similar success to that obtained using fatty acid profiles (Table 3). Although the classifying performance using sensor and fatty acid data was not validated against a separate data set (the sample size was not enough), these results suggest that suitable fat classification using the electronic nose is possible. Because the sensor responses occur as a result of the interaction of sensors with volatile compounds, some consideration should be taken into account to prevent uncontrolled lipid oxidation. Processing method and storage conditions should be the same for all the samples, as would be required to analyze volatile compounds.

This electronic nose provides suitable results and could be used as a feasible alternative to the cumbersome fatty acid analysis for the classification of samples according to their fatty acid composition. Moreover, it could be useful for on-line controls because it is easier and faster to use.

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REFERENCES

1. Flores, J., C. Birón, L. Izquierdo, and P. Nieto, Characterization of Green Hams from Iberian Pigs by Fast Analysis of Subcutaneous Fat, *Meat Sci. 23*:253–262 (1988).
2. Ruiz, J., R. Cava, T. Antequera, L. Martín, J. Ventanas, and C.L. López-Bote, Prediction of the Feeding Background of Iberian Pigs Using the Fatty Acid Profile of Subcutaneous, Muscle and Hepatic Fat, *Ibid. 49*:155–163 (1998).
3. Eklöv, T., G. Johansson, F. Winquist, and I. Lundström, Monitoring Sausage Fermentation Using an Electronic Nose, *J. Sci. Food Agric. 76*:525–532 (1997).
4. Gardner, J.W., and P.N. Bartlett, *Electronic Noses: Principles and Applications*, Oxford University Press, Oxford, 1999, pp. 140–183.
5. García, C., J.J. Berdagué, T. Antequera, C. López-Bote, J.J. Córdoba, and J. Ventanas, Volatile Components of Dry Cured Iberian Ham, *Food Chem. 41*:23–32 (1991).
6. López, M.O., L. De la Hoz, M.I. Cambero, E. Gallardo, G. Reglero, and J.A. Ordóñez, Volatile Compounds of Dry Hams from Iberian Pigs, *Meat Sci. 31*:267–277 (1992).
7. Shen, N., S. Duvick, P. White, and L. Pollak, Oxiadative Stability and AromaScan Analyses of Corn Oils with Altered Fatty Acid Content, *J. Am. Oil Chem. Soc. 76*:1425–1429 (1999).
8. Young, H., K. Rossiter, M. Wang, and M. Miller, Characterization of Royal Gala Apple Aroma Using Electronic Nose Technology-Potential Maturity Indicator, *J. Agric. Food Chem. 47*:5173–5177 (1999).
9. Carrapiso, A.I., M.L. Timón, M.J. Petrón, J.F. Tejeda, and C. García, In Situ Transesterification of Fatty Acids from Iberian Pig Subcutaneous Adipose Tissue, *Meat Sci. 56*:159–164 (2000).
10. Green, S.B., N.J. Salkind, and T.M. Akey, *Using SPSS for Windows: Analyzing and Understanding Data*, Prentice Hall, Saddle River, New Jersey, 1999, pp. 170–270.
11. Sammon, J.W., A Nonlinear Mapping for Data Structure Analysis, *IEE Transactions on Computers C.18 (5)*:401–409 (1969).
12. Hair, J.F., R.E. Anderson, R.L. Tatham, and W.C. Black, *Multivariate Data Analysis*, 5th edn., Prentice Hall, New Jersey, 1998, pp. 87–314.
13. Bishop, C.M., *Neural Networks for Pattern Recognition*, Oxford University Press, Oxford, 1995, p. 130.
14. Christie, W.W., D. McEwan Jenkinson, and J.H. Moore. Variation in Lipid Composition Through the Skin and Subcutaneous Adipose Tissue of Pigs, *J. Sci. Food Agric. 23*:1125–1129 (1972).
15. Romain, A.-C., J. Nicolas, V. Wiertz, J. Maternova, and P. André, Use of a Simple Tin Oxide Sensor Array to Identify Five Malodors Collected in the Field, *Sensors Actuators B 62*:73–79 (2000).
16. Haugen, J.E., and K. Kvaal, Electronic Nose and Artificial Neural Network, *Meat Sci. 49*:S273–S286 (1998).
17. Rocha, S., Y. Delgadillo, A.J. Ferrer Correia, A. Barros, and P. Wells, Application of an Electronic Aroma Sensing System to the Cork Stopper Quality Control, *J. Agric. Food Chem. 46*:145–151 (1998).

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