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Table olives volatile fingerprints: Potential of an electronic nose for quality discrimination

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Abstract

In the present work, the potential of an electronic nose to differentiate the quality of fermented green table olives based on their volatile profile was investigated. An electronic gas sensor array system comprising of a hybrid sensor array of 12 metal oxide and 10 metal ion based sensors were used to generate a chemical fingerprint (pattern) of the volatile compounds present in olives. Multivariate statistical analysis and artificial neural networks were applied to the generated patterns to achieve various classification tasks. Green olives were initially classified into three major classes (acceptable, unacceptable, marginal) based on a sensory panel. Multivariate statistical approach showed good discrimination between the class of unacceptable samples and the classes of acceptable and marginal samples. However, in the latter two classes there was a certain area of overlapping in which no clear differentiation could be made. The potential to discriminate green olives in the three selected classes was also evaluated using a multilayer perceptron (MLP) neural network as a classifier with a 18-15-8-3 structure. Results showed good performance of the developed network as only two samples were misclassified in a 66-sample training dataset population, whereas only one case was misclassified in a 12-sample test dataset population. The results of this study provide promising perspectives for the use of a low-cost and rapid system for quality differentiation of fermented green olives based on their volatile profile.

Keywords: Table olives; Sensors; Quality; Electronic nose; Volatile fingerprints; Machine learning; Neural networks

1. Introduction

Odour is a major olfactory parameter determining the sensory quality of food products and it is therefore of interest to investigate if volatile compounds contributing to the characteristic odours can be measured as indicators of quality assessment. This is also important for green table olives, one of the most popular fermented vegetables in the Western world. The production of volatile compounds tends to be the first mechanism for the development of flavour specific to a particular fermented food. Lowering the pH in lactic acid fermentation may reduce the enzymatic activity in olive tissue that generate either flavour components or flavour precursor compounds. Additionally, the microorganisms during the course of fermentation may directly metabolise precursor flavour compounds of flavour components producing a plethora of volatile compounds (esters, acids, alcohols, aldehydes, ketones, phenols, etc.) that characterise the process [1].

Generally, sensory analysis based on a trained expert panel is necessary for table olive classification and quality control but it is not always feasible due to high cost, while it is time-consuming and often without any objective value. In the last decades many efforts have been made to study the aromatic fraction of fermented olives based mainly on chromatographic determinations [2-9]. However, these analytical techniques are also time-consuming and require sophisticated equipment and skilled personnel. It would be therefore of great interest to investigate the potential of low-cost, rapid and non-destructive analytical procedure, such as the electronic nose, to quantify the overall quality of table olives.

In the last decade the electronic nose technology has offered the possibility to exploit, from a practical point of view, the information contained in the headspace in many different application areas. The use of the electronic nose for quality evaluation as a means of non-destructive olfactory sensing is becoming widespread as it has the advantage of low cost, good reliability and high portability for *in situ* and on-line measurements [10,11]. Moreover, this technique has been shown to be rapid and simple compared with GC-MS [12-14]. The underlying hypothesis in electronic nose sensing for food safety/quality assessment is that the interaction of microbial association with the food system affects the type as well as the rate of metabolites produced either in the form of gas, solid or liquid. Sensing the gaseous metabolites (mainly volatile organic compounds) present in the headspace of a food commodity could provide useful information for the determination of the quality of a given food product [15]. The interaction of volatiles on the sensing element causes changes in electrical resistance of the sensor, and since sensor kinetics is different, the data produced are converted into an odour fingerprint which can be interpreted with the use of appropriate mathematical techniques, like multivariate statistical methods or artificial neural networks (ANNs). The obtained data are comparable in the sense that different samples may be characterised and discriminated based on their volatile production patterns [16,17]. In the literature there are several reports that demonstrate the potential of electronic nose in food-relevant applications for the classification of vegetable oils [18], quality control of olive oil aroma [19], characterisation of wines [20,21], determination of fish freshness [22], quality estimation of ground meat [23], grain quality evaluation [15,24-26], detection of microorganisms [27-30], detection of boar taint in meat [31], shelf life determination of tomato [32], and quality assessment of modified atmosphere packaged poultry meat [33]. However, the application of an electronic nose for quality discrimination of fermented table olives is reported here for the first time.

An artificial neural network (ANN) can be referred to as a neurocomputer with parallel distributed processors [34]. ANNs have been employed in recent years as an alternative to conventional regression models, due to their ability to describe highly complex and non-linear problems in many fields of science. The most common neural network approach is multilayer perceptrons (MLPs) [35]. The basic idea behind ANNs lies in the direct exploration of the knowledge contained in the input-output patterns by adjusting the parameters of the non-linear network topology, as the input-output patterns are repeatedly presented to the network [34]. When the system is supervised using an appropriate training dataset it can then be used to predict or classify different patterns not initially introduced to the network.

The objective of this study was to evaluate the performance of an electronic nose system to differentiate the quality of fermented green table olives based on their volatile fingerprints and classify them in three major classes (acceptable, marginal, unacceptable). Classification of the samples was based on both multivariate data analysis and artificial neural networks (ANNs) algorithms.

2. Materials and methods

a. Table olive samples

Twenty six samples of fermented green olives were obtained from a central hyper-market in Athens. The amount of each sample was *ca.* 0.5 kg. The volatile profile was each sample was assessed by a sensory panel and classified into three major groups, namely unacceptable (n = 3), acceptable (n = 16), and marginal (n = 7). As there was no previous information on the application of an electronic nose for table olive quality assessment based on their volatile profiles, a bottom-up approach was followed to create a knowledge basis for the product. Initially, the potential of electronic nose to discriminate between unacceptable and a typical acceptable (reference) sample was investigated. Furthermore, the analysis was extended taking into account the unacceptable and marginal samples together with the reference sample. In the final step, all groups of samples were taken together and their volatile profile was recorded and subjected to multivariate analysis.

b. *Electronic nose data acquisition and analysis*

Electronic nose analysis was performed with a NST 3320 Lab Emission Analyser (Applied Sensors, Linköping, Sweden), equipped with a built-in headspace autosampler for 12 samples, a detector unit containing 23 different sensors and a built-in software package (NST Senstool) for collecting and processing data from the sensors. The instrument was equipped with 12 MOS (metal oxide semiconductor) sensors operating at 250-400°C, 10 MOSFET (metal oxide semiconductor field effect transistor) sensors operating at 140-170°C, and a humidity sensor for the determination of relative humidity at 70°C. The electronic nose operating system employed in this study has been described in detail elsewhere [33].

Olive samples of 2 g were weighed and placed in 25 ml glass vials, sealed with septa and screw caps, and incubated at 37°C for 1 h for headspace equilibration above the sample. Samples were then randomly placed in the autosampler and headspace gas was pumped over the sensors of the electronic nose. The total cycle time per sample was 4 min and 20 s. The baseline phase was set to 30 s, the sample phase to 30 s and the recovery phase of the sensors to 240 s including the flush time of the gas lines . Every sample was analysed in triplicate.

c. *Data analysis*

The data collected were analysed using the XLStat software (version 2006.06, Addinsoft, Paris, France), a built-in statistical software package of Excel. Data exploration and interpretation was based on multivariate analytical techniques such as principal components analysis (PCA), hierarchical cluster analysis (HCA), and discriminant function analysis (DFA) on the obtained sensor responses. Prior to analysis the raw sensor responses were normalised using the following equation:

$$X_{norm} = \frac{X_i - X_{\mathbf{min}}}{X_{\mathbf{max}} - X_{\mathbf{min}}} \tag{1}$$

where, X_i and X_{norm} are the raw and normalized response of a sensor *i*, and X_{min}, X_{max} are the minimum and maximum sensor responses of the *i* sensor, respectively.

Predictive learning (classification) was performed on the whole dataset (n = 78) of unacceptable, acceptable and marginal samples using artificial neural network (ANN) techniques. The type chosen in this study was a multilayer perceptron (MLP) based on back propagation. In these networks each node receives signals through connections with other nodes or the outside world in the case of the input layer. The net input to node *j* has the form:

$$I_j = \sum_{i=1}^{n} w_{ij} \cdot x_i + \theta_j \tag{2}$$

where x_i are the inputs (sensor responses in our case), w_{ij} are connection weights associated with each input/node and θ_j is the bias associated with node *j*. The output from each node is used as an input in a nonlinear transfer function:

$$O_j = f\left(I_j\right) \tag{3}$$

In our work the sigmoidal function was selected as a transfer function in both hidden and output neurons. The standard backpropagation algorithm for network training is based on the steepest-descent gradient approach applied to the minimization of the error function defined as:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}\left(t_i - d_i\right)^2 \tag{4}$$

where t_i represents the desired network output, d_i is the actual network output, and n is the number of samples. The difference between the desired value and the actual network output was propagated back through the network to the input level. This training process, known as delta rule, is based on the minimization of the error by adjusting the weights given by the following equation:

$$w_{kj}\left(n+1\right)=w_{kj}\left(n\right)+\eta e_k\left(n\right)x_j\left(n\right)+\alpha\Delta w_{kj}\left(n\right) \quad (5)$$

where $w_{kj}(n)$ is the weight adjustment at time n, e_k is the negative derivative of the total square error with respect to the neuron's output, x_j is the element of the input vector, η is the learning rate parameter, and α the momentum term. In order to train and test the developed neural network, the entire database (78 samples) was divided into training (66 samples) and test (12 samples) dataset. To avoid any bias in database partitioning, table olive samples were allocated into each category using a method based on random number generation. The strategy employed for the classification of olives is the so-called one-of-many encoding [36]. The output of the network is a multidimensional vector with the number of the dimensions equal to the number of the classes of table olives to be determined, and each vectorial dimension is assigned to a class. In the training file, the class membership of a single data is coded in a numerical format by assigning 1 to the belonging class and 0 to all others, i.e., acceptable olives are coded as (1,0,0), marginal samples as (0,1,0), and unacceptable as (0,0,1). In the test file, the membership of an input data is assigned to the class with greatest net output. The prediction performance of the network was defined as the ratio of number of correctly identified patterns to that of total patterns introduced to the network. The MLP network was developed using NeuralWorks Professional II/PLUS version 5.50 (NeuralWare, Carnegie, PA).

3. Results

Analyses were initially conducted with all samples of unacceptable olives together with the reference sample and the headspace of three replicates per treatment was examined using metal oxide/ion-based sensor arrays for discrimination. Replication of the sensor array was firstly examined and was found to be very consistent (Figure 1), indicating clear differences in volatile fingerprints between unacceptable and reference samples. The variation between replicates was relatively small and consistent. The potential for discrimination was better in the MOS sensors rather than the MOSFET sensors. Four of the sensors (MOSFET 104B, 105B, MOS 115, 116) of the electronic nose were excluded due to limited or very high response, when subjected to the volatile compounds of green olives. In addition, the responses of the humidity sensor were excluded as they did not show any clear differences among the samples.

Figure 2 depicts a PCA scores plot of the separation between the samples. This shows that PC1 and PC2 accounted for almost 91.6% of the variance and resulted in a clear discrimination between the volatile patterns of unacceptable samples compared to the reference sample. Cluster analysis, using Euclidean distance and Ward's linkage measure, on the same dataset, using just the first two principal components, also produced a similar result (Figure 3). Two major groups can be visualised with reference samples (A) being separate from unacceptable ones (HN, PL, AV).

Figure 4 shows a PCA scores plot of the separation between unacceptable, marginal and the reference sample. About 97.4% of the total variance of the data was explained by PCA in which PC1 and PC2 accounted for 87.1% and 10.3%, respectively. Although a clear differentiation was evident between the reference sample and the unacceptable and marginal samples as a whole, no clear separation could be obtained between the latter two groups. However, after analysing the same dataset with DFA each group was clearly distinguishable (Figure 5). The two discriminant functions accounted for 99.7% of the variance, indicating that the separated result is better with DFA (supervised method) than PCA (unsupervised method). It is worth noting that when the same data set was analysed with both MOSFET and MOS sensors no clear differentiation could be obtained among the groups (data not shown). For this reason, only the responses of the MOS sensors were taken into account for further analysis, as sample separation could be better attained with these types of sensors (see Figure 1). The data presented in Figure 6 is the whole dataset consisting of unacceptable, marginal and acceptable samples. DFA analysis showed good discrimination between unacceptable samples and the other two qualitative groups of green olives. However, in the case of marginal and acceptable samples there was an area of overlap between the two clusters (area of uncertainty), implying that some samples cannot be classified as belonging clearly to one class or the other.

The MLP neural network based on back propagation was used to classify olive samples into the three categories from the volatile metabolites profile obtained from the electronic sensor array analysis. For neural network development three steps were followed including creating the network, training the network, and validating the network. A variety of hidden layers (one or two), hidden neurons, learning rates and momenta were tried by developing different networks. The selected network architecture included an input layer, two hidden layers of neurons, and an output layer. There were 18 neurons in the input layer (one for each sensor apart from the initially excluded sensors), 15 neurons in the first hidden layer, 8 neurons in the second hidden layer, and 3 neurons in the output layer (Figure 7). The learning rate (n = 0.10) and momentum (α = 0.20) parameters were selected to ensure that the convergence of the learning process was achieved. The learning process performed until the error covering the entire training dataset converged to a minimum value (RMSE = 0.017), whereas the relative value of RMSE for the test dataset was 0.206. Two olive samples were misclassified in the training dataset. Specifically, one marginal sample was classified as acceptable, and one unacceptable sample was classified as acceptable. In the test dataset, one sample was misclassified, i.e. an unacceptable sample was classified as acceptable (Table 1). The prediction performance of the network was fairly high as 97% and 92% for the training and test dataset, respectively.

4. Discussion

This study examined the potential of using an array of metal oxide/ion sensor system for differentiating the quality of fermented green olives. Results indicated that qualitative volatile patterns using electronic nose technology could be successfully employed as a rapid tool for table olives discrimination. The volatile fraction of table olives depends on many factors such as variety, ripening stage, process conditions, and the microbiological composition of the fermentation brines [37]. Several classes of compounds have been identified in the aroma profile of table olives, the most important being ethanol, methanol, 2-butanol, acetone, ethyl acetate, and acetaldehyde for both green and black olives [9, 38]. Many of these volatile compounds are among the end products known to be formed by the initial (e.g. coliforms, yeasts, etc) and/or final microbial association e.g. lactic acid bacteria, the major group responsible for olive fermentation or any other group (e.g. yeasts, filamentous fungi, clostridia) associated with spoilage of these fermented products [2]. It is thus clear that volatile compounds have a decisive role in the characterisation of the flavour pattern of a given olive sample. Sensory quality is taken into great account by consumers, and is therefore the predominant element of appreciation and choice.

Indeed sensory analysis has evolved considerably in recent years thanks to the development of statistical analysis techniques which are crucial for data processing. However, although taste panels are useful in quality classification tasks they have high running costs, are time consuming, and sometimes lead to controversial interpretations. Therefore it would be interesting to employ another method for quality discrimination based on instrumental analytical techniques, such as the electronic nose. As a first step in the investigation of the method, the volatile patterns of samples characterised as unacceptable by a sensory panel were compared with an acceptable sample that was considered as a reference. The electronic nose could clearly distinguish between the two classes as inferred by principal components analysis (PCA) and cluster analysis (CA) (see Figures 2, 3). However, as more samples were introduced in the analysis, PCA, as an unsupervised method, could not produce satisfactory discrimination (see Figure 4). For this reason, discriminant function analysis (supervised method) was employed producing a clear differentiation between unacceptable, marginal and reference sample (see Figure 5). An electronic nose is a system created to mimic the function of human nose. However, this analytical instrument is more a multi-sensor array technology than a real nose. Therefore, a sensory panel is necessary to define the desired product quality which in turn can be used to train the system for maximum discrimination [39]. Finally, when all samples were analysed and subjected to multivariate statistical interpretation, a certain level of overlapping between the classes of marginal and acceptable samples was observed (Figure 6). As discriminant function analysis was employed in the differentiation of the samples, this overlapping could be due to misclassification of the original samples by the sensory panel.

The discrimination of the analysed table olive samples has been tackled with a pattern recogniser based on an artificial neural network providing nonlinearity in the multivariate classification performance. A feed-forward fully connected multilayer perceptron network has been trained with the back-propagation algorithm for this purpose. By introducing nonlinear methods like ANNs, it would be possible to model electronic nose data in a better way, as multivariate statistical techniques are based on a linear approach, neglecting the fact that gas sensor array data may often be nonlinear in nature [35]. The overall performance of the predictive recogniser can be appreciated by the confusion matrix obtained for each one of the three sensory classes used (see Table 1). One of the three misclassified olive samples lies in the zone of uncertainty, as it was originally marginal and classified as acceptable. For the other two points, probably a larger training dataset or another neural network classifier (e.g. a radial basis function network) could have improved classification performance [26].

5. Conclusion

The results of this study demonstrated the applicability of an electronic nose as a screening tool for quality control of fermented table olives based on their volatile patterns. It is worth noting that better description of the quality classes is necessary by an expert sensory panel to improve the discriminating performance of the system. Even if a larger number of table olive samples is required for better discrimination, the present results demonstrated the possibility of using this analytical technique to obtain in a rapid and objective manner information about the sensorial properties of table olives.

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Figure Legends

Figure 1. Comparison of the responses of metal oxide sensors to three replicates of unacceptable olive samples (solid line) and the acceptable reference olive sample (dotted line).

Figure 2. The principal components (PC) analysis map of data for three replicates of unacceptable olive samples and the acceptable reference sample using the metal oxide/ion-based electronic nose.

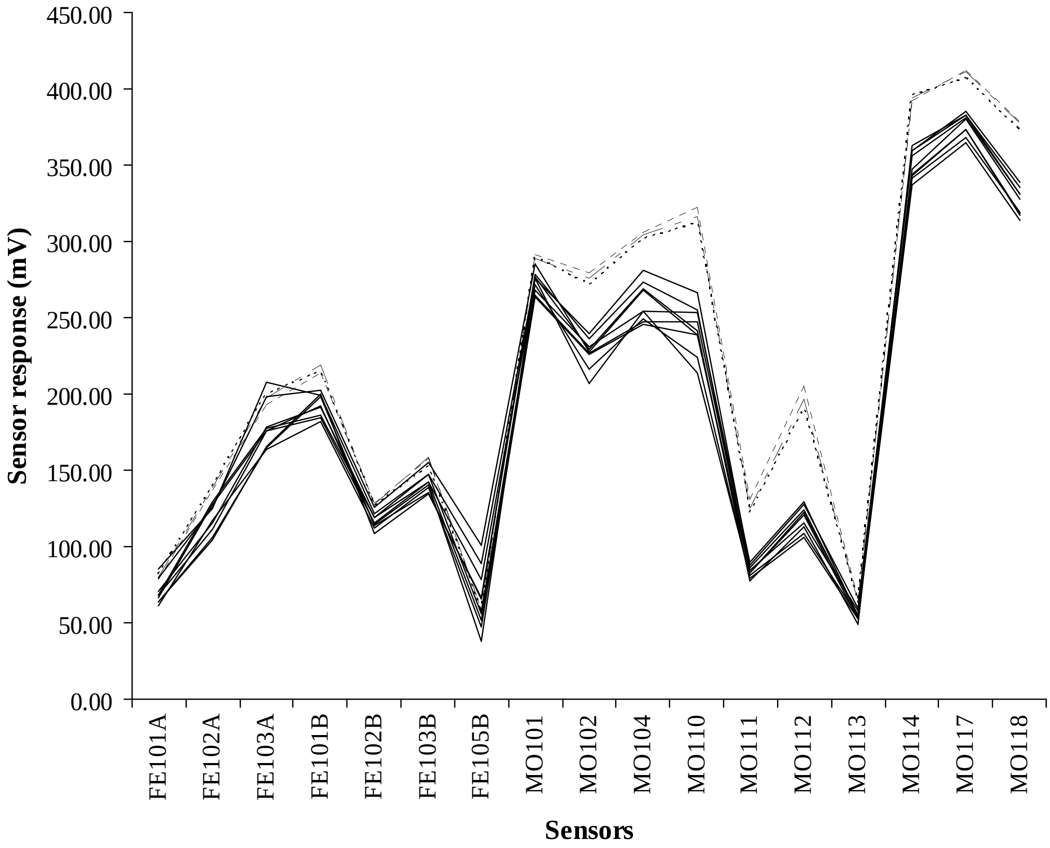
Figure 3. Dendrogram showing the cluster analysis and separation of the acceptable reference sample (A) and the unacceptable olive samples (AV, PL, HN). Numbers correspond to the replicates for each olive sample.

Figure 4. The principal components (PC) analysis map of data showing the discrimination of unacceptable (U), marginal (M) and the acceptable (A) reference sample. Three replicates for each sample were used.

Figure 5. Discriminant function analysis (DFA) plot of the differentiation between unacceptable, marginal and the acceptable reference sample. Three replicates for each sample were used.

Figure 6. Discriminant function analysis (DFA) plot of the differentiation between unacceptable, marginal and acceptable olive samples. Three replicates for each sample were used.

Figure 7. Schematic representation of a four-layered MPL network used as patterns classifier for table olives quality identification.


450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Sensor response (mV)
FE101A
FE102A
FE103A
FE101B
FE102B
FE103B
FE105B
MO101
MO102
MO104
MO110
MO111
MO112
MO113
MO114
MO117
MO118
Sensors

1

2 Fig. 1

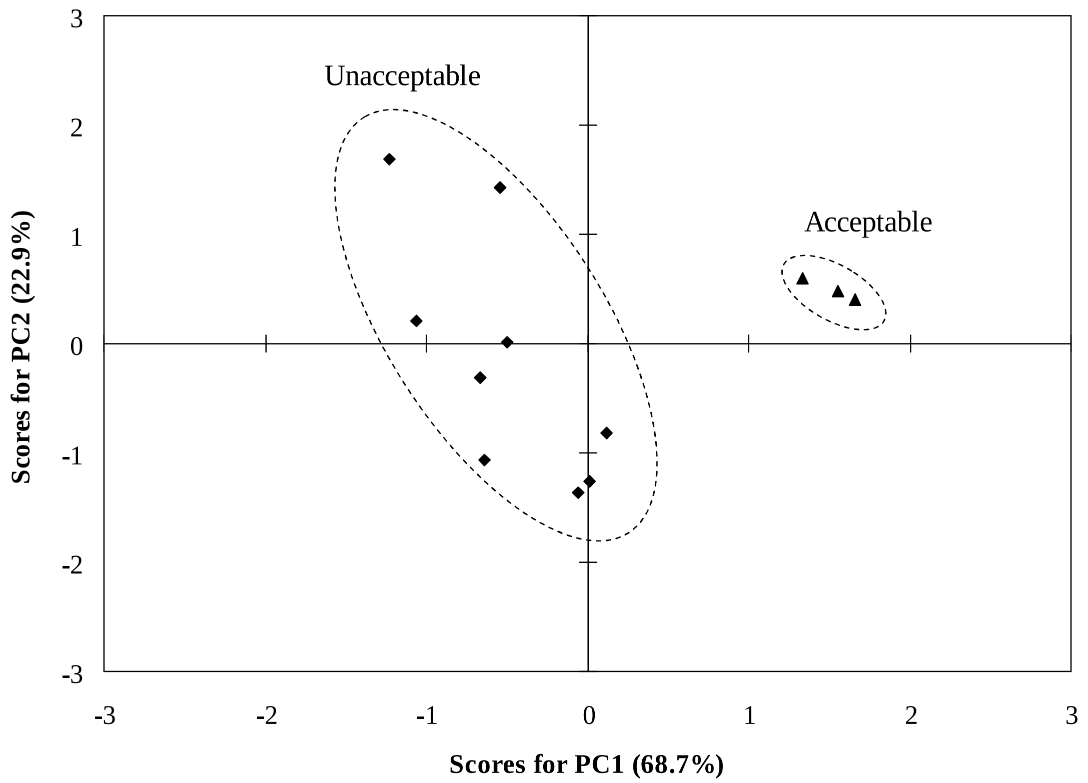

Unacceptable
Acceptable
Scores for PC2 (22.9%)
Scores for PC1 (68.7%)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3

Fig. 2

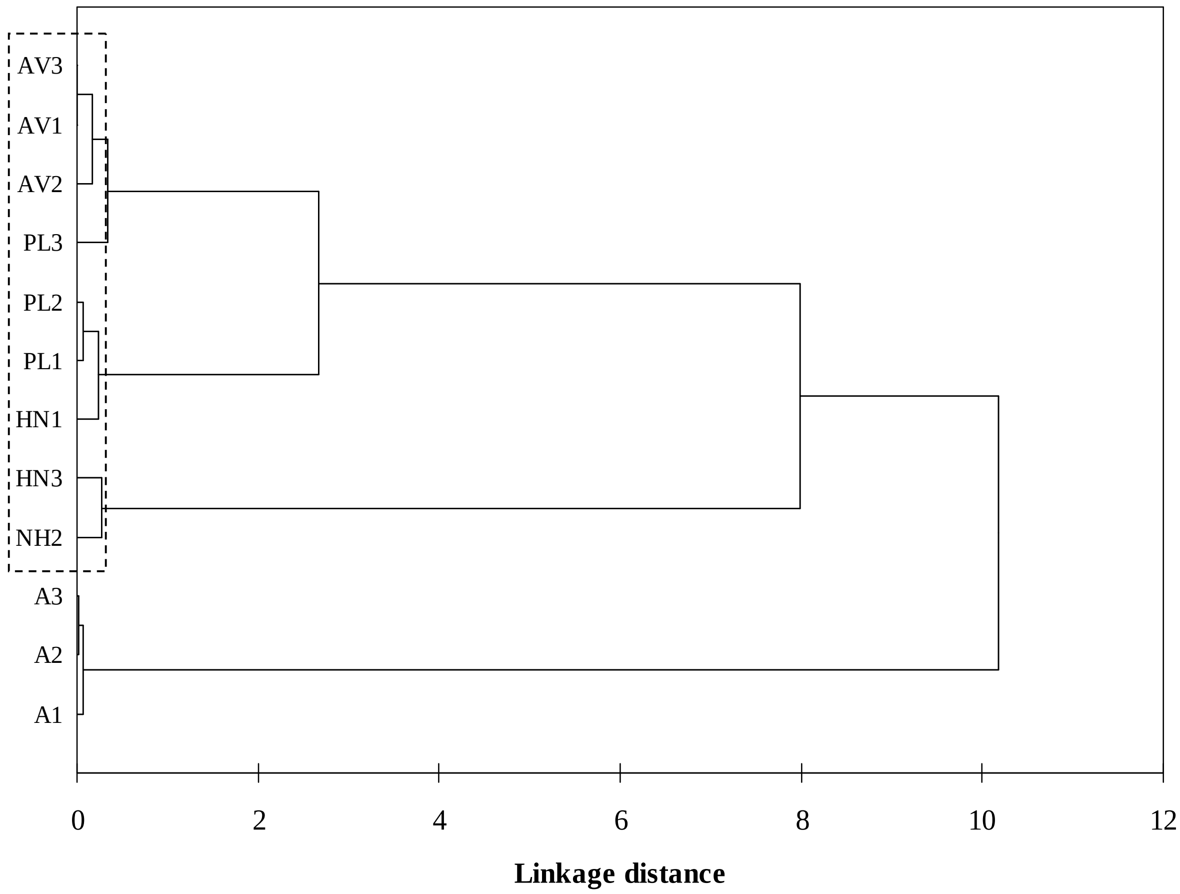
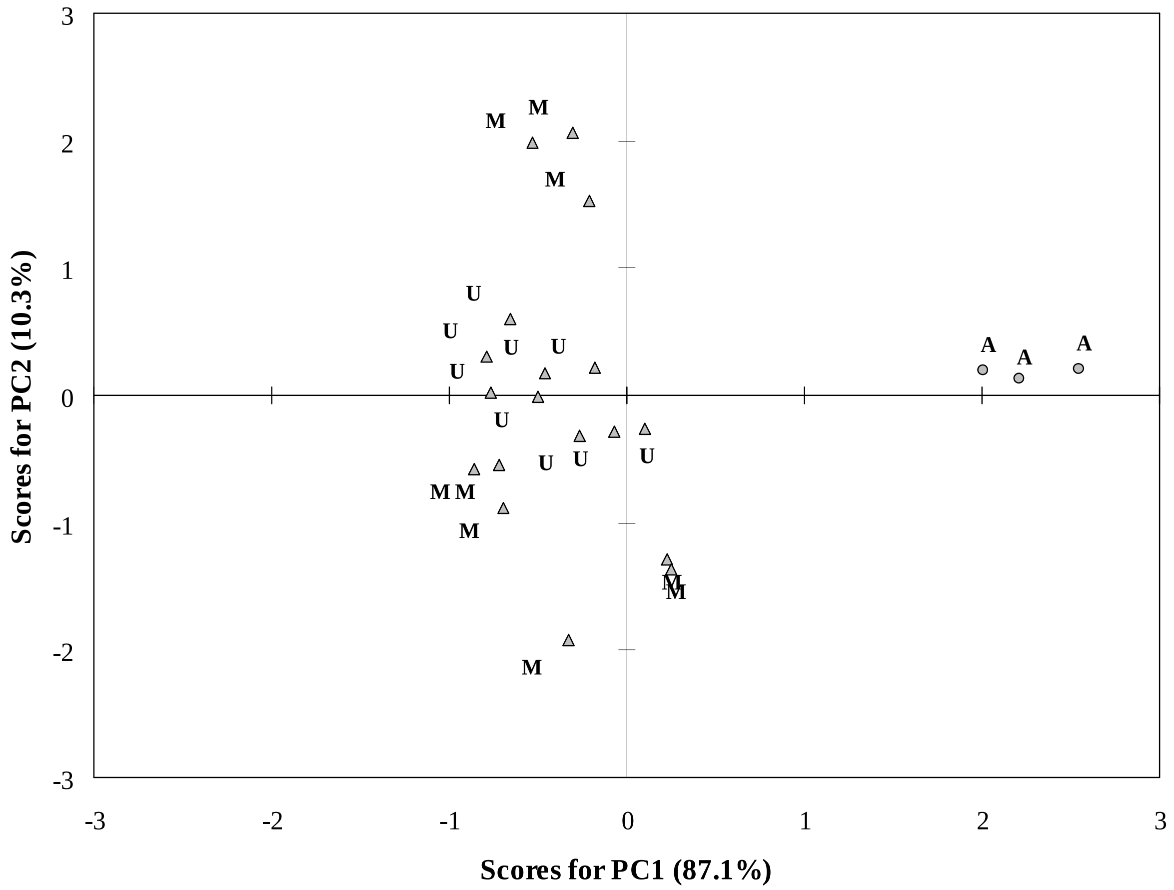

AV3
AV1
AV2
PL3
PL2
PL1
HN1
HN3
NH2
A3
A2
A1
0
2
4
6
8
10
12
Linkage distance

Fig. 3


Scores for PC2 (10.3%)
Scores for PC1 (87.1%)
3
2
1
0
-1
-2
-3
-3
-2
-1
0
1
2
3
M
M
M
U
U
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U
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U
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M M
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M
M
M
A
A
A

Fig. 4

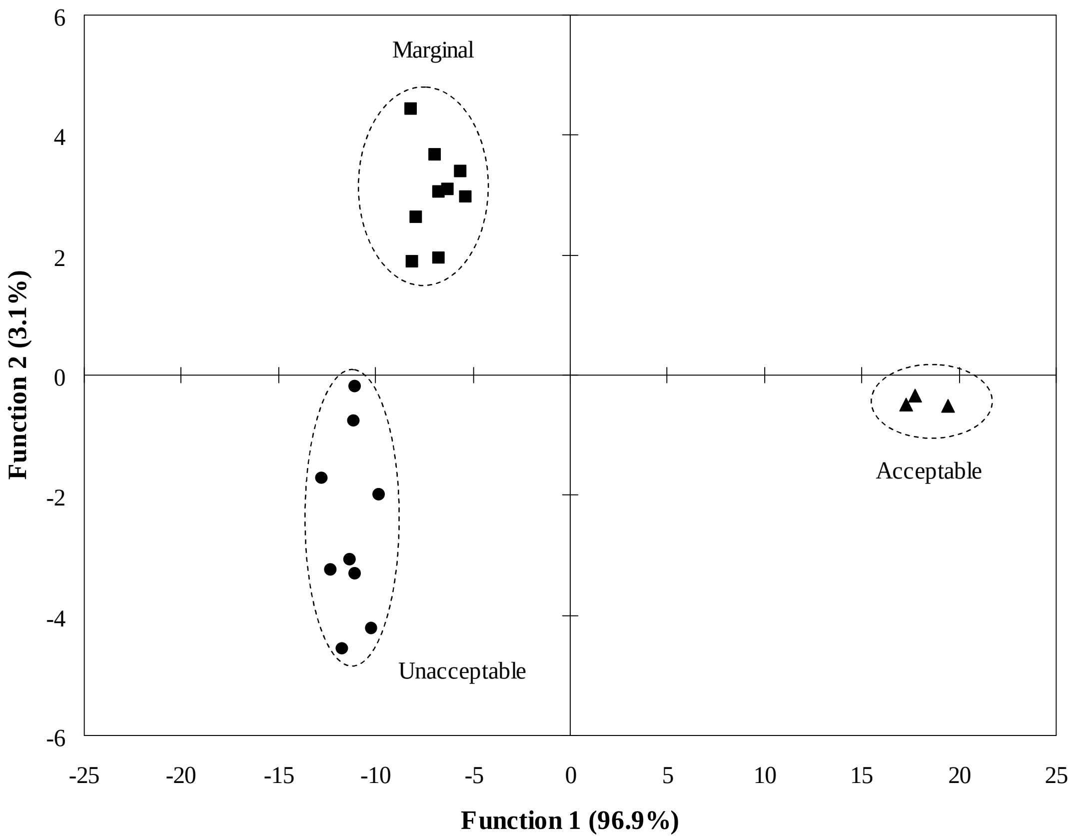
Marginal
Unacceptable
Acceptable
Function 2 (3.1%)
Function 1 (96.9%)
6
4
2
0
-2
-4
-6
-25
-20
-15
-10
-5
0
5
10
15
20
25

Fig. 5

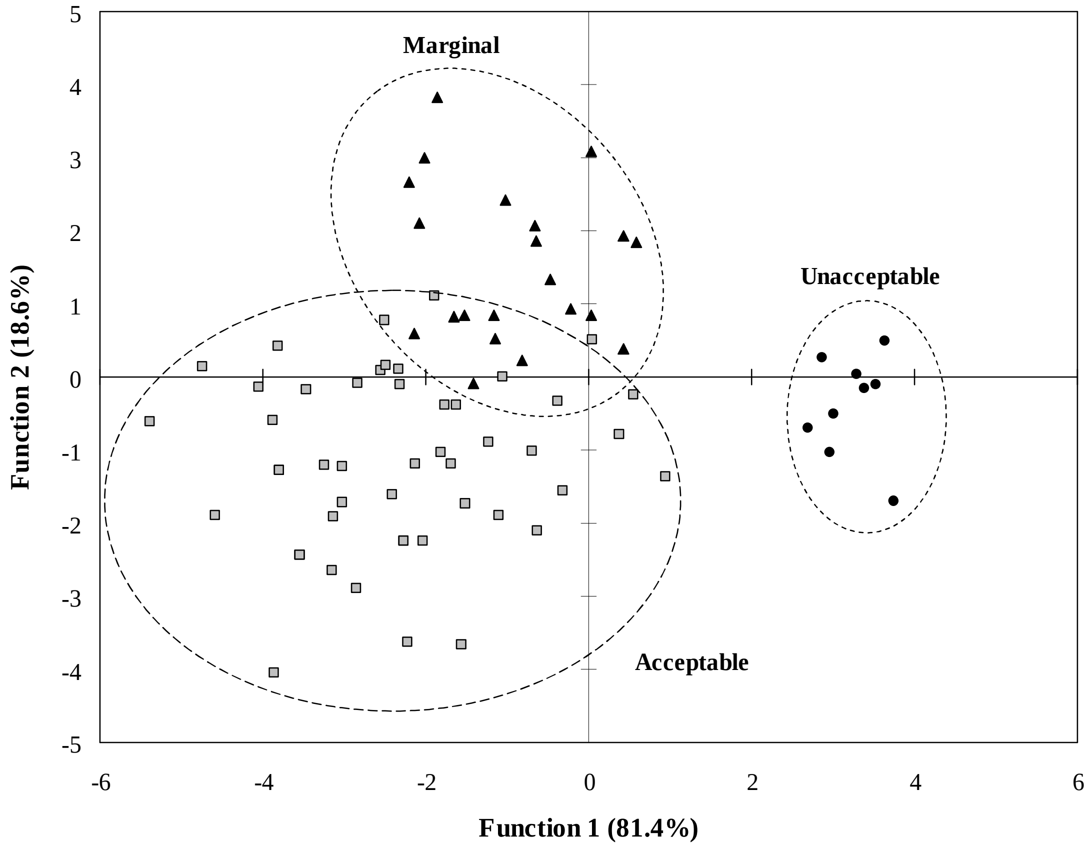
Marginal
Unacceptable
Acceptable
Function 2 (18.6%)
Function 1 (81.4%)
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-4
-2
0
2
4
6

Fig. 6

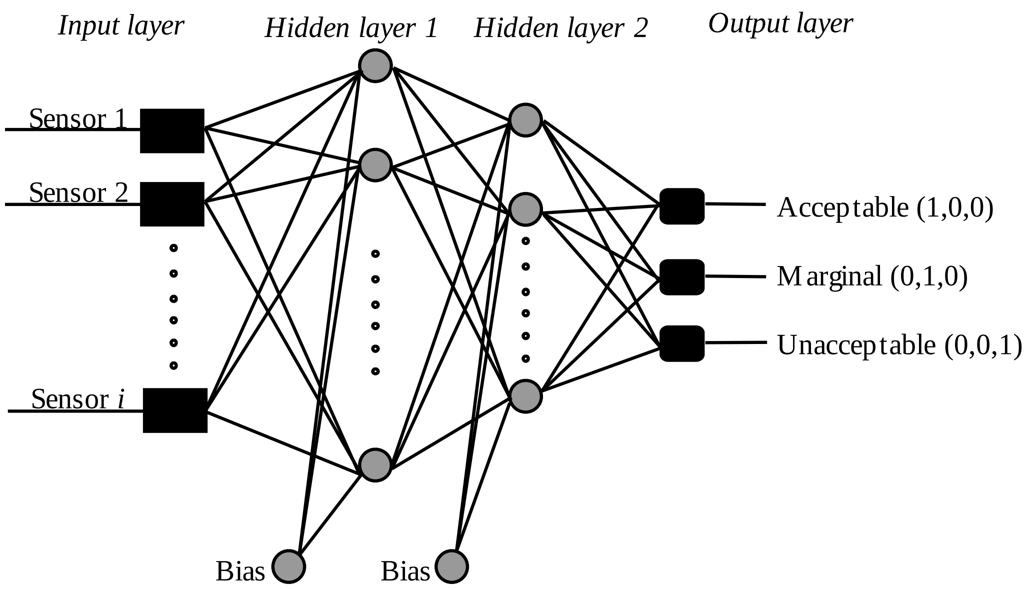
Input layer
Hidden layer 1
Hidden layer 2
Output layer
Sensor 1
Sensor 2
Sensor i
Bias
Bias
Acceptable (1,0,0)
Marginal (0,1,0)
Unacceptable (0,0,1)

Fig. 7

Table 1

Confusion matrix of the MLP classifier performing the task of discrimination of table olive samples.

	From/To	Acceptable	Marginal	Unacceptable
Training dataset	Acceptable	34	0	0
	Marginal	1	17	0
	Unacceptable	1	0	13
Test dataset	Acceptable	5	0	0
	Marginal	3	0	0
	Unacceptable	1	0	3

Table olives volatile fingerprints: Potential of an electronic nose for quality discrimination.

Panagou, Efstathios Z.

2008-09-25

E.Z. Panagou, N. Sahgal, N. Magan, G.-J.E. Nychas, Table olives volatile fingerprints: Potential of an electronic nose for quality discrimination, Sensors and Actuators B: Chemical, Volume 134, Issue 2, 25 September 2008, Pages 902-907

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