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Using an "electronic nose" to discriminate white truffle (*Tuber magnatum* Pico) quality

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RÉSUMÉ

Utilisation d'un « nez électronique » pour la discrimination de la qualité de la truffe blanche (*Tuber magnatum* Pico).

Avec un nez artificiel électronique et un panel d'experts on a analysé cinquante et un échantillons de truffe blanche *Tuber magnatum* Pico. Le but de cette expérimentation a été de voir s'il y avait une relation entre l'odeur perçue par le nez artificiel et la « qualité » indiquée par ce panel. Le nez artificiel utilise des capteurs à MOS et MOSFET. Ces derniers sont beaucoup plus performants que les capteurs précédents à cause de leur sensibilité supérieure et surtout parce qu'ils ne sont pas influencés par l'humidité à différence du MOS. Avec l'analyse discriminante et les réseaux de neurones on a obtenu une très bonne discrimination entre les truffes et il est donc possible d'utiliser ce nez artificiel électronique pour classer les truffes en fonction de leur valeur commerciale après une phase d'apprentissage très approfondie. Il y a eu aussi des erreurs de classification, mais ces erreurs ont été faites parce que les experts ont utilisé avec l'odeur aussi la couleur, la structure et la taille, que le nez artificiel ne peut pas évaluer. On peut donc supposer que le nez artificiel, après un bon apprentissage, peut être utilisé avec le panel d'experts pour une évaluation des truffes plus objective.

Mots clés : *« nez électronique », capteurs à oxydes métalliques, truffe blanche, analyse discriminante linéaire, réseau de neurones.*

SUMMARY

Fifty-one white truffle (*Tuber magnatum* Pico) samples were examined by a commercial "electronic nose" and by an expert panel. The aim was to define a relationship between the truffle odor perceived by electronic sensors and the "quality" evaluation furnished by this panel. The instrument, which uses MOS and MOSFET sensors, is particularly advanced in respect to previous models and the data furnished have allowed optimal repeatability in the responses. The best results are given by the MOSFET sensors which are less influenced by

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sample humidity than the MOS sensors. Using Linear Discriminant Analysis and Artificial Neural Networks, the correct classification of more than 80% of the truffle samples was possible. The wrong classification of any samples was due to the fact that the experts used not only odor, but other evaluation parameters such as colour, structure and dimension which the electronic nose could not note. It can therefore be hypothesized that after a suitable phase of training, the electronic nose can be integrated with the panel in order to improve the objectivity of the evaluations.

Key-words: *"electronic nose", metal oxide sensors, white truffle, Linear Discriminant Analysis, Artificial Neural Networks.*

1 – INTRODUCTION

Truffles are subterranean fungi which grow in mycorrhizal association between tree roots and the mycelium. The white truffle (*Tuber magnatum* Pico) is one of the most important and valued truffles in the world for its unique and intense aroma and is found mainly in Italy. Its commercial value is very high, approximately $2500 per kg, and as a consequence there are frequent cases in which lower "quality" and hence lower value species of truffle found in other countries are sold as Italian *Tuber magnatum*. Therefore there is a need for consumer protection which guarantees that the place of origin and the truffle "quality" correspond to those declared.

There are many studies on the volatile components (TALOU *et al.*, 1987; TALOU, 1989; TALOU *et al.*, 1989a, b, c; FLAMENT *et al.*, 1990; TALOU *et al.*, 1990; MAC-IARELLO and TUCKER, 1993; PELUSIO *et al.*, 1995; BELLESIA, 1998; BELLESIA *et al.*, 1996, 1998; BELLESIA *et al.*, 1997; SALTRON *et al.*, 1997; WOOD *et al.*, 1998) and DNA (GANDEBOEUF *et al.*, 1994; HENRION *et al.*, 1994; POTENZA *et al.*, 1994; CALLOT *et al.*, 1996; MELLO *et al.*, 1996; AGOSTINI *et al.*, 1997; ARCIONI *et al.*, 1997; PAOLOCCI *et al.*, 1997; BERTAULT *et al.*, 1998; BERTINI *et al.*, 1998; RUBINI *et al.*, 1998; ROSSI *et al.*, 2000) of the more important truffle species (*Tuber magnatum, T. melanosporum, T. brumale, T. aestivum*) and with these works it is possible to distinguish the truffle's origin and the storage effects, but the analytical procedures proposed are long, expensive, and above all require sampling of the truffles themselves and therefore damaging them.

Moreover these procedures are inapplicable for rapid checks on the large amounts of the product sold in markets or fairs.

Until now truffle "quality" evaluation has always been done by highly experienced experts. However, often, the expert's opinions are subjective.

More than the truffle's appearance (form, dimension and colour), evaluation is based above all on its odour intensity and on the absence of extraneous odours. Replacing this human evaluation with a sensory system or "electronic nose" which can quickly test the acceptability and the value of the truffles is therefore desirable and possible.

The principle of its operation is clearly distinct from the conventional analytical instruments (GC, GC-MS, HPLC, etc.). In fact it does not analyse the food volatile fraction by separating and identifying the various components, but it tests the intensity of this volatile fraction and its components. Moreover, it can be used on high value samples, for which it is not possible to use any technique which damages or simply requires the handling of the sample.

There are numerous examples of electronic nose applications for testing foods and for the differentiation of origin and production technology (AISHIMA, 1991; GARDNER *et al.*, 1992; MOY, 1992; PEARCE *et al.*, 1993; GARDNER *et al.*, 1994; ZANNONI, 1995; BÖRJESSON *et al.*, 1996; DI NATALE *et al.*, 1996; BARTLETT *et al.*, 1997; DI NATALE and D'AMICO, 1997; JONSSON *et al.*, 1997; PORRETTA *et al.*, 1997; BAZZO *et al.*, 1998; JOU and HARPER, 1998; KESHRI *et al.*, 1998; MAUL *et al.*, 1998; SBERVEGLIERI *et al.*, 1998; SCHALLER *et al.*, 1998; SCOTT, 1998; SPAGNOLI and PORRETTA, 1998; VISSER and TAYLOR, 1998; WIJESUNDERA and WALSH, 1998; CHATONNET, 1999; MARSILI, 1999; NITZ *et al.*, 1999; RIDGWAY *et al.*, 1999; ZONDERVAN *et al.*, 1999). The electronic nose has also been applied to truffles but only for evaluating the effects of storage and for determining the best harvesting time (MOY *et al.*, 1991). The aim of this work is to verify whether or not the electronic nose is able to discriminate the same "quality" of the white truffles as perceived by an expert.

2 – MATERIALS AND METHODS

The electronic nose used in this study was a semi-conducting metal oxide based on a gas sensor array developed by Nordic Sensor Technologies, Sweden (NST version 3210). The sensor array consisted of one block with ten MOSFET and one block with five commercial chemical sensors based on tin dioxide, also known as Taguchi sensors or MOS-type sensors. The sensors were set at an operating temperature between 150 and 400°C. Ambient air filtered by active charcoal was used as the carrier gas with a flow rate of 170 $mL \cdot min^{-1}$. The same carrier gas was used to transfer the sample headspace into the sensors. The total cycle time for each measurement was set for 5mn as follows: 10 s baseline, 60 s sampling and 230 s air recovery. The sampling rate was one point per second. The truffles to be examined were placed in glass containers with metal screw-on lids fitted with perforated silicone membranes through which the automatic sampler needle withdraws the sample from the headspace.

Two experiments were made. The aim of the first was to test the instrument reproducibility with truffles. Four samples were tested: a *Tuber mesentericum*, a *Tuber melanosporum*, a *Tuber borchii* and a *Tuber magnatum*. Each sample was analysed 16 times with 30 mn intervals between each analysis.

In the second test the electronic nose was tested as a potential instrumental technique to predict the commercial value of white truffles. Fifty-one samples of *Tuber magnatum* were tested. Three measurements were made for each sample. The truffles botanical classification was made by the Mycological Institute of Turin (Italy) while their commercial value was assessed by a panel formed by ten experts and five "truffle finders". Consumer tests were not possible with white truffles because of their very high value.

The panel expressed its judgement synthetically by qualifying to each product examined either "Good" or "Bad".

The signals obtained from each sensor (*figure 1*) shows a curve formed by an initial ascent corresponding to the aspiration phase of the sample headspace and a descent relative to the sensor cleaning phase.

The rising and descending slopes as well as the maximum intensity of the signal depend on the substance examined and on the sensor considered.

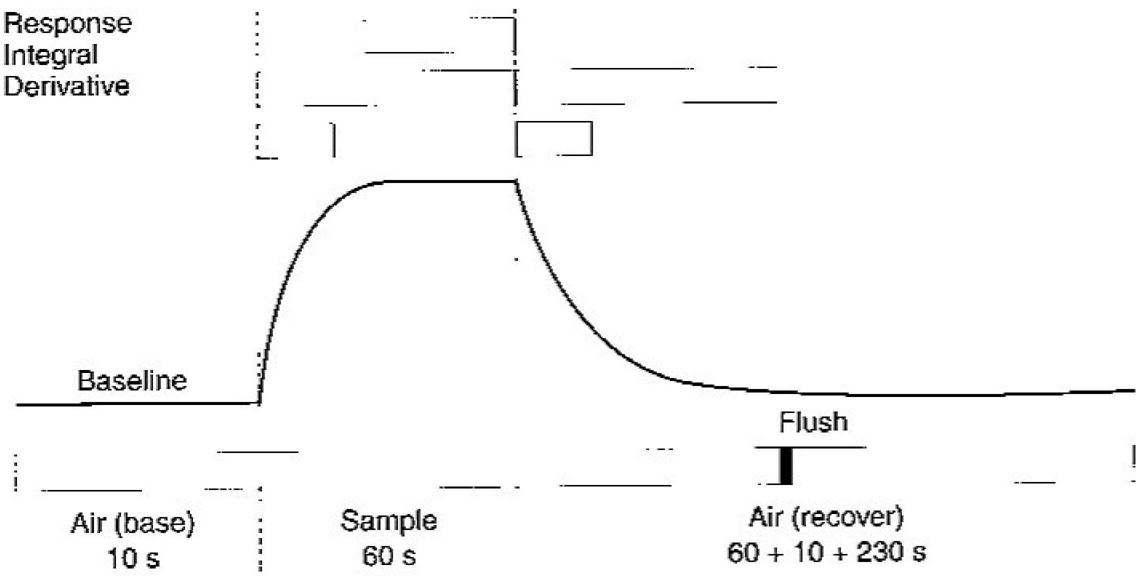

Figure 1

Signals obtained from the electronic nose sensors and related parameters

Hence the same sensor will be able to yield different curves for different substances and this difference will influence one or more of the curve parameters (initial and final slope and maximum value).

From this signal it is then possible to obtain five parameters: maximum intensity of the response, the first derivative and the integral of the rising phase, the first derivative and the integral of the descending phase.

The two integral values supply more information about the curve because two segment slopes are summarized (the rising and the descending ones) and the maximum intensity of the sensor response.

In this work therefore the values of the integral calculated for the signal rising and the integral calculated for the signal descending phase were used for each sensor.

The integral values were computed for each curve obtained by the MOSFET and MOS-type sensors for the 51 white truffles examined and each repetition was subjected to multidimensional statistical processing with Senstool ver. 2.5f (NST, Sweden), Statistica for Windows ver. 5.5 (StatSoft Inc., OK, USA) and NeuroShell 2 (Ward System Groups, Frederick, MD, USA). The procedures used were Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and Artificial Neural Networks (ANNs).

A PCA was used for a data multidimensional scaling and a sample description with only two dimensions. The PCA was applied only in the repeatability study. A LDA was used instead to verify whether or not a relationship existed between the two truffle "qualities", those the experts determined "Good" and "Bad", with the data furnished by the electronic nose. LDA is a supervised statistical processing tool and is considered an important classical parametric method for grouping samples when the sample allocation is previously known and for this reason it has been successfully used for the geographical differentiation and classification of various foods. However, for predictive purposes it is a technique of scarce interest. To allow recognition and then the exact classification of unknown samples with some previously measured reference data, artificial neural networks (ANNs) can be created. The ANNs are a powerful tool for characterising and discriminating food products, originally intended as a model of the human neural system. ANNs have been applied in many scientific fields: physics, medicine, botany and food science (DAWN, 1994; VALLEJO-CORDOBA *et al.*, 1995; GERBI *et al.*, 1998; NI and GUNASEKERAN, 1998; CICHELLI *et al.*, 2000; MITTAL and ZHANG, 2000). ANN is a computed system which processes numerical input (training data) through a learning process and converts it to final output. An ANN is typically organized in "layers" which are made-up of a number of interconnected "nodes". Usually the data (in this case the integral value of the sensor signals) are passed to ANN via the "input layer" and with a system of "transfer function" and "node weights" the network computes a response at the output layer. These values are then compared to the actual values of the response variable (in this case the expert judgement) and an error is computed. With a backpropagation algorithm this error is used to adjust the "node weights" and the network computes a new response. The iterative procedure of processing inputs, determining the errors and adjusting the weights is the "learning process". Iteration continues until the network's response errors are kept to a minimum. In this work the ANNs were constructed by using the integral values as input neurons and the two truffle categories as output neurons. The network architecture used was a the three-layer, fully interconnected, feed-forward type. They were constructed with a learning rate of 0,1 and a momentum of 0,1. To avoid network overtraining, NET-PERFECT™ was used. This is an implemented procedure of NeuroShell 2 that creates an entirely separate set of data, called test-set, and uses it to evaluate how well the network is predicting. NET-PERFECT™ was used to compute the optimum point for saving the network when it is able to generalise new data well. In particular, the network learning was carried out with a limit of 200.000 events after the test set minimum mean value of re-classification error was reached.

3 – RESULTS AND DISCUSSION

3.1 Repeatability of measurements

The variation coefficient calculated for each sensor and for each sample for the integral values is between 1% and 12% and is therefore similar to that reported by CHATONNET and DUBOURDIEU (1999) for MOS sensors. However these authors operated with the response intensity and thus with a better signal stability. Such low variation coefficients for the integral values indicate a high instrumental repeatability in the headspace aspiration phase and in the sensor cleaning and also a high stability of the same sensors. The sensor signals which vary in time have a greater coefficient variation than those with random variations.

Generally the MOS sensors have variation coefficients greater (on average 10%) than MOSFET ones (on average 6%). The MOSFET sensors low sensibility to humidity plays an important role in the signal stability (NST Internal Report).

The stepwise-PCA applied to the integral of sensor curves computed two factors which explain 94% of the variance. The score plot with the 16 analyses for the four truffles is reported in *figure 2*. The truffle aroma is then very stable at room temperature for at least 8 h and does not appear to be affected by the repeated headspace sampling.

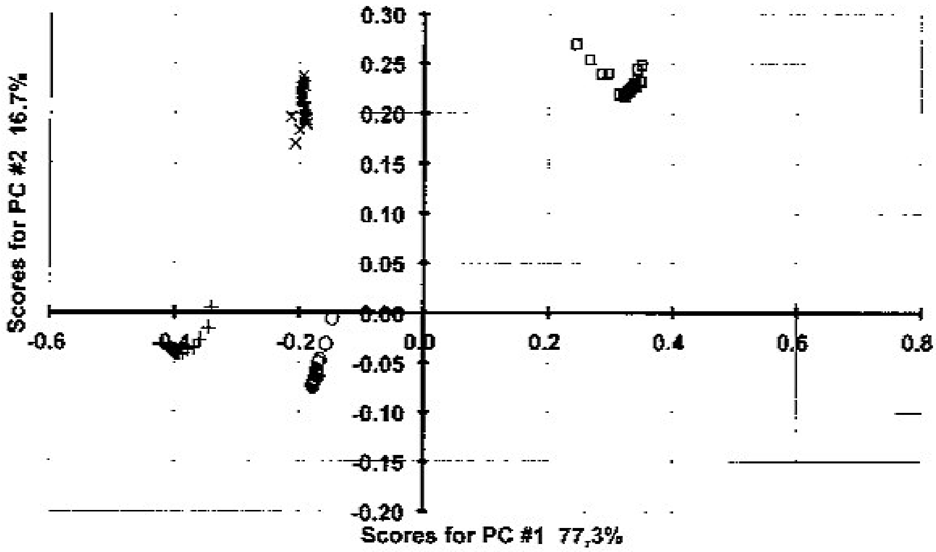

Figure 2

Score plot of the truffles tested

+ *T. mesentericum*; × *T. melanosporum*; ☐ *T. magnatum*; ○ *T. borchii*.

3.2 Discriminating truffle "quality"

Of the 51 examined samples 27 were judged "Good" and 24 "Bad" by the expert board. In *figure 3* the parameter profiles (response, first derivative and integral) for all sensors of a good and a bad truffle are reported. Wilk's lambda (PIGGOTT, 1986) for the computed model of LDA with the "Standard method" is 0.3 and therefore the two clusters are well split. In fact the classification exceeds 85% and the Mahalanobis distance between the "Good" and "Bad" groups is 4.75.

The mean score value for the "Good" group is 0.68 while that of the "Bad" cluster is – 1.47 (*figure 4*). Therefore the discrimination is very good and the partial overlap between the two clusters regards mostly outlying samples.

The judgement expressed by this panel takes into account visual and tactile aspects as well, that, obviously the electronic nose cannot account for in its evaluation and this explains the classification errors.

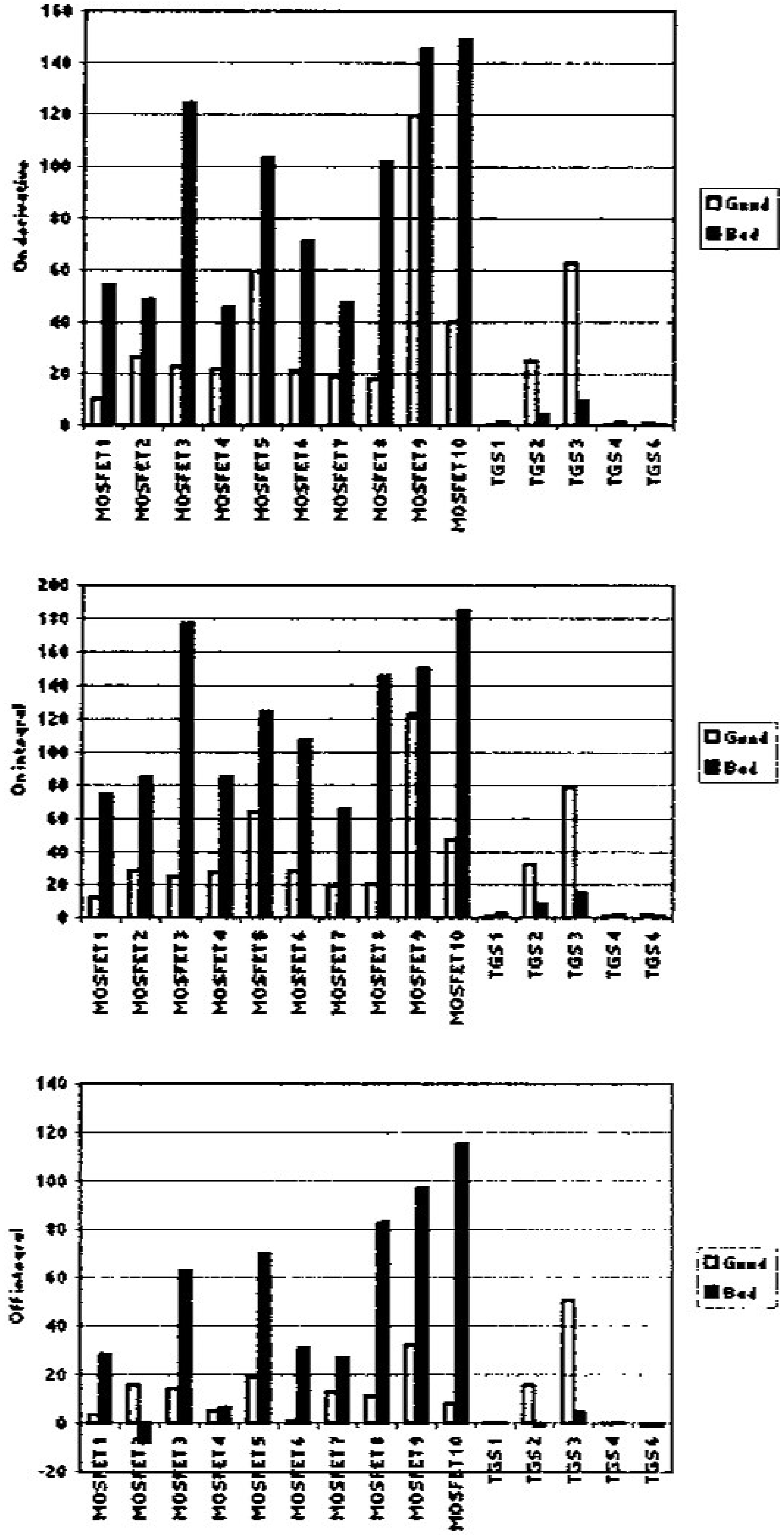

Figure 3

"Good" ◻ and "Bad" ■ truffle profiles of the five parameters computed for the signal sensors

TGS: Taguchi sensor.

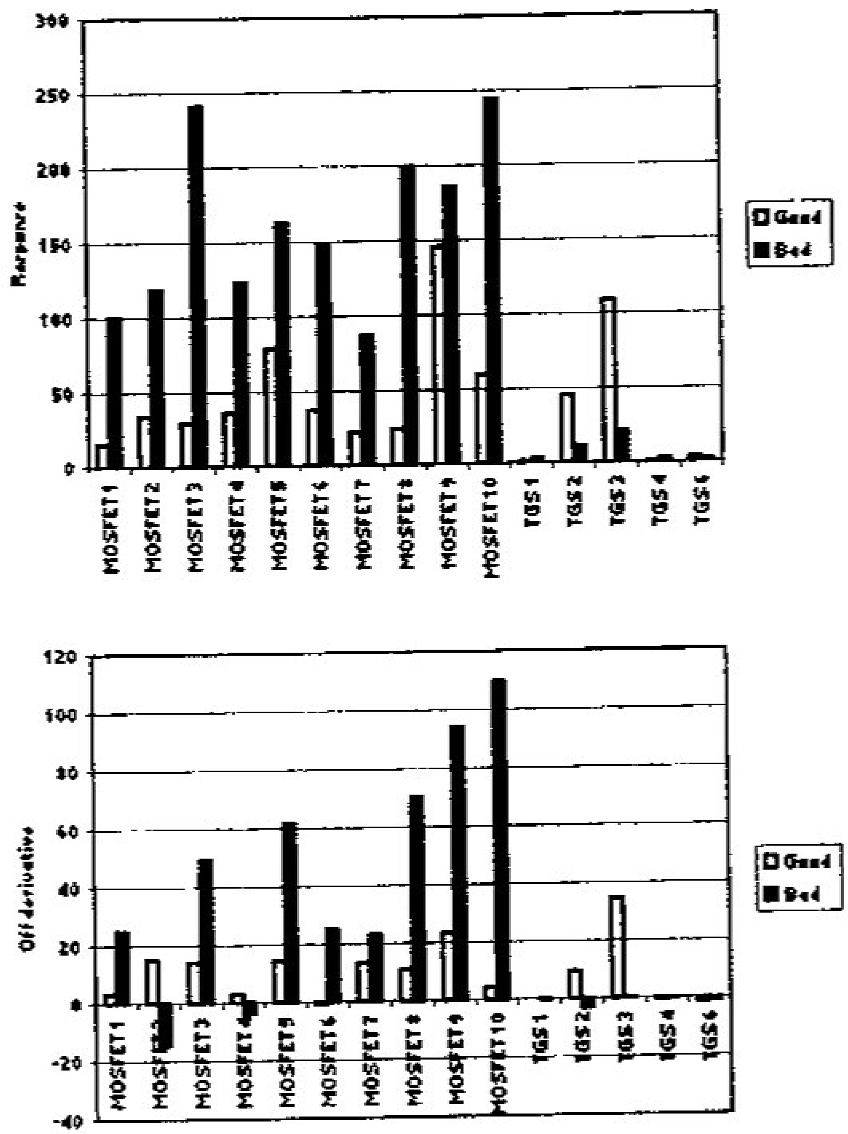

Figure 3 (continued)

"Good" □ and "Bad" ■ truffle profiles of the five parameters computed for the signal sensors

TGS: Taguchi sensor.

3.3 Neural Network Prediction

For ANNs, all the 153 analyses (51 samples, three repetitions) were used. These samples were randomly subdivided into a training set (80%) and a validation set (20%). In the training set, therefore, there are 123 analysis and in the validation one 30. The distribution percentage of the samples between the two data sets was chosen empirically and represents a compromise between the need to have the maximum number of samples in the training set while at the same time representing both product categories in the validation set. The ANN construction process, from the two data set extractions through learning, was repeated ten times. The ANN parameters used for the truffle classification are shown in *table 1*. On the other hand *table 2* presents the average learning network generalisation levels. Besides using 30 input neurons, the ANN was formed with 27 hidden neurons. Variation in the hidden neuron number did not

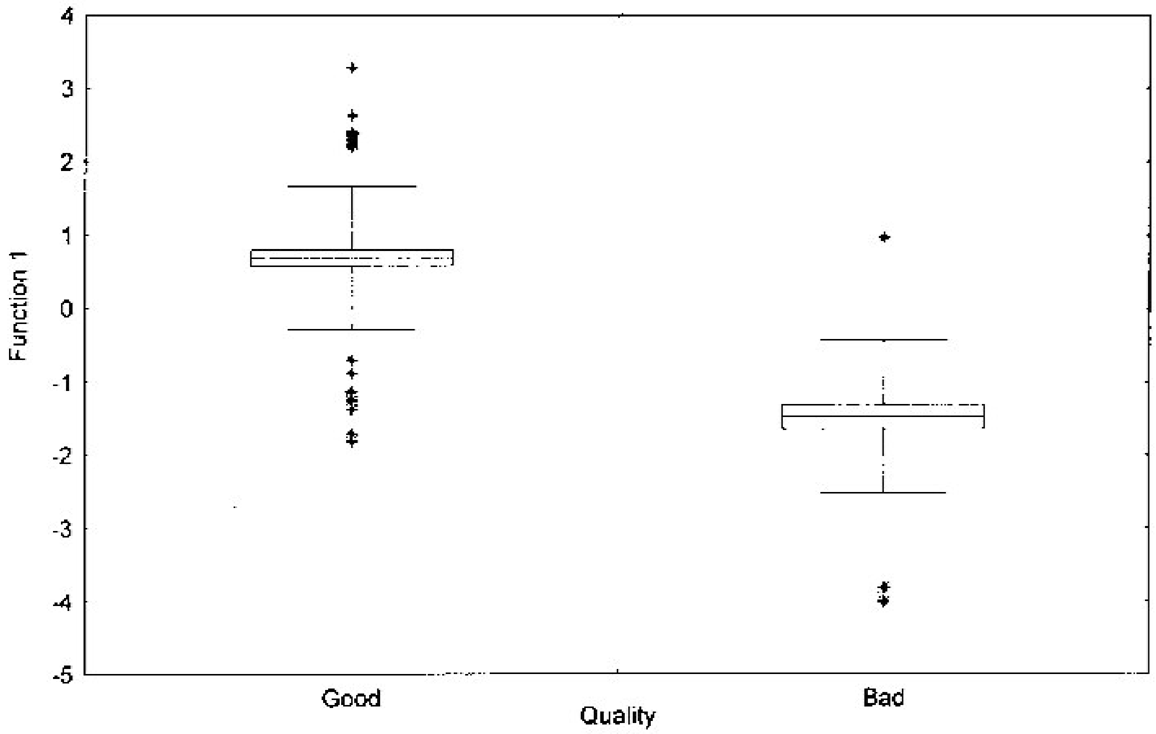

Figure 4

Box-plots of truffle sample scores computed with a LDA

influence the network learning level. Likewise, the variable selection for their contribution factors did not influence the network learning level.

The ANN results show that the classification was well defined and only eight samples (four in the training set and four in the validation set) are misclassified. These classification errors are due to the experts' use of other parameters (colour, structure, dimension) in their truffle evaluation.

Table 1

Parameters of the ANN

Events since min average error	200 000
Learning events	2 179
Learning rate	0.1
Momentum	0.1
Training data	
Minimum average error	0.002806
Maximum average error	0.03921
Correlation	0.946
Validation data	
Minimum average error	0.1099
Maximum average error	0.1882
Correlation	0.946

Table 2

Average learning and artificial network generalization levels

		Number of cases	Correctly classified cases	Wrongly classified cases
Training set	Number	123	119	4
	%		97	3
Validation set	Number	30	26	4
	%		88	12

4 – CONCLUSIONS

The electronic nose has been shown to be a reproducible instrument also in the analysis of the white truffle or *Tuber magnatum* Pico. It is able to differentiate between different "quality" samples and their indications usually coincide with those of the experts. The best results are furnished by the MOSFET sensors which are less influenced by sample humidity than the MOS sensors. The wrong classification of any samples was due to the fact that the experts used not only odour, but other evaluation parameters such as colour, structure and dimension which the electronic nose could not note. It can therefore be hypothesized that, after a suitable phase of training, the electronic nose can be integrated with the evaluating panel, leading to more objective evaluations.

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