Olfactometric techniques in feed analysis: preliminary calibration of DON in durum wheat

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RIASSUNTO – Approcci olfattometrici nell'analisi dei mangimi: calibrazione preliminare per il rilievo di DON nel grano duro. *Le analisi olfattometriche, attraverso l'uso del naso elettronico, trovano sempre più spazio nella valutazione della qualità e sicurezza in campo alimentare. I principali vantaggi di tale approccio risiedono nella sua flessibilità di impiego. Una volta messi a punto adeguati protocolli d'analisi e modelli statistici di tipo multivariato, infatti, il naso elettronico si può rivelare uno strumento adatto alle realtà di campo in quanto caratterizzato da rapidità e basso costo d'esercizio. Lo scopo di questo lavoro è stato quello di verificare, a livello preliminare, l'efficienza del naso elettronico nel riconoscere la presenza di micotossina deossinivalenolo (DON) in 10 campioni di grano duro* (Triticum durum) *di diversa provenienza geografica. I risultati hanno dimostrato che il protocollo applicato consente di riconoscere la presenza di DON nonché di discriminare i diversi campioni in funzione del loro livello di contaminazione.*

Key words: electronic nose, olfactometric techniques, DON, durum wheat.

INTRODUCTION – Electronic nose (EN), among the most recent olfactometric techniques, represents a modern and automated analytical approach which could have many industrial applications in different fields. Food analyses represent one of the more interesting examples. One of the most throughput definitions of the EN, was given by Gardner and Bartlett (1994), who defined it as "an instrument which comprises an array of electronic chemical sensors with partial specificity to chemical substances and an appropriate pattern recognition (PR) system, capable of recognizing simple or complex odours". The possibility to avoid or strictly reduce sample pre-treatment and to test different kind of simple or complex matrixes, are two of the most important features of this kind of assay. For these reasons, EN enables to reduce test time and labour both in laboratory and industrial process control, such as food industry applications (Feast, 2001). The PR is a sort of *smell print*, which can be used to discriminate sample by their aroma. The application of chemometric techniques (multivariate analysis such as training and prediction algorithms) to data processing, enables to extract information used for designing odour classes. Thus, even if the EN sensors are not explicitly sensible to specific chemical substances, it is possible to classify samples in classes according to a selected attribute of quality, such as conservation or chemical/microbial contamination, etc.
Since the successful application of olfactometric techniques as fast and cost–effective approach for food authenticity, quality and safety assessment, the aim of this study was to evaluate applicability of EN to feed analysis, particularly to mycotoxins detection in durum wheat (*Triticum durum*).

MATERIAL AND METHODS – 10 durum wheat (*Triticum durum*) samples of different geographical origin were collected according to 98/53/CE, 2002/26/CE and 2002/27/CE Directives. Each sample was split into

two sub-samples. The former was analysed by HPLC assay for aflatoxins, ochratoxin A and trichothecenes content, the latter was submitted to olfactometric analysis. 2 g of each sample was hermetically sealed in 12 ml vials and heated 5 min at 40°C for temperature equilibration. The head space of each sample was aspirated for 300 sec and sent to EDU2 Enricher/Desorber (Air sense Analytics GmbH, Sherwin, Germany) equipped with a column containing TENAX® adsorbent resin, which traps organic volatiles and concentrate them (enrichment). The volatiles adsorbed by the resin are then released through thermic desorbtion at 200°C for 180 sec and injected for 40 sec into the EN. The odours profile were therefore determined by the 10 MOS (Metal Oxide Semiconductor) sensors of the PEN2 EN (Air sense Analytics GmbH, Sherwin, Germany). Conducibility of each sensor was recorded by the EN software with one second frequency and the last 4 seconds of sampling (when the 10 signals were stable) were finally considered for statistical analysis.
The data obtained were subsequently submitted to principal component analysis (PCA) and principal component regression (PCR) by means of the SAS procedure PROC PRINCOMP and PROG REG (SAS, 2001). Cross validation of the statistical model was finally performed by means of "one at a time" method of PROC PLS procedure (SAS, 2001) which extracts each sample and let the statistical model predict its contamination by the others.

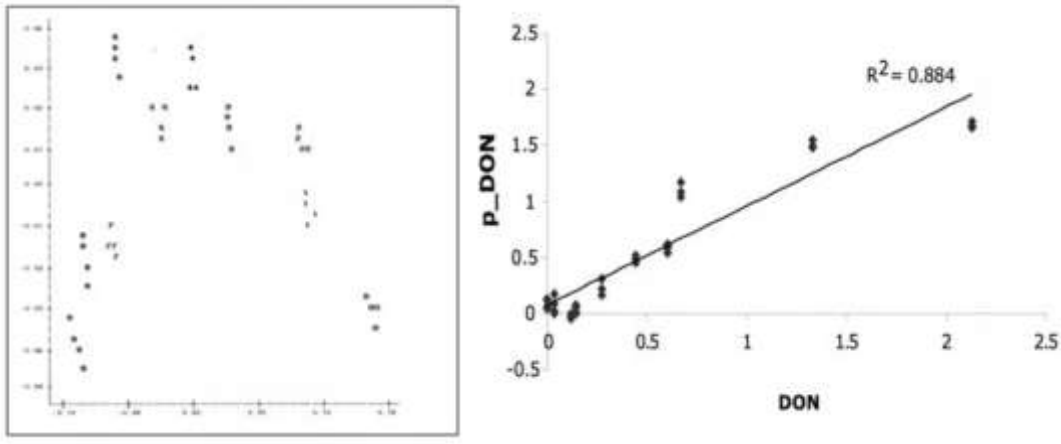
RESULTS AND CONCLUSIONS – HPLC results showed that samples were free from aflatoxin (<0.1 mg/kg), ochratoxin A (<1 mg/kg) and fumonisins contamination. Trichotecens were absent too (<0.001 mg/kg), except for DON. Contamination levels for each sample are listed in table 1.

Table 1. DON content in durum wheat samples.

Sample	DON (mg/kg)
0 - Italy (Biocorato)	< 0.001
1 - South Australia	0.035
2 - Spain	0.120
3 - Australia (Kronos)	0.144
4 - Italy (Borgo Libertà)	0.274
5 - North Australia	0.442
6 - Italy (mix 2)	0.604
7 - Italy (mix 1)	0.670
8 - France	1.330
9 - North Dakota	2.130

Data obtained from Enricher/Desorber and EN combined analysis were submitted to principal component analysis. The results indicate that the 99.45% of the total variability was explained by the two first principal components (corresponding respectively to sensors "broad-alcohol", 90.41% of total variability, and "aromatic 1", 9.04% of total variability). The graph reported in figure 1 represents the distribution of the two first principal components: the samples were numbered from 0 to 9 according to their level of contamination (0 for the wheat sample negative for DON; 9 for the sample with highest level of contamination). Figure 1a shows clear patterns proportional distribution according the DON concentration explained by the first principal component.
Data were then submitted to principal component regression. The activation of the two first principal components correlated significantly with the level of DON contamination (R^2=0.866; P<0.0001).
The results were finally validated by means of the "one at a time" method, which extracts each sample and let the statistical model predict its contamination by the others. The predicted values of DON contamination were then correlated with HPLC results (Figure 1b) and showed a significative correlation (R^2=0.88).
Previous experiments (Tognon *et al.*, 2004) performed without the use of Enricher/Desorber on the same samples group, showed only a partial ability of EN alone to discriminate wheat samples, because a lot of samples overlapped in PCA graph. The experience here described, demonstrates that the use of Enricher/Desorber

Figure 1. (a) Results of principal component analysis; (b) Correlation between predicted (p DON) and true (DON) DON concentrations for each wheat sample.



enables rather a clear discrimination of different level of DON contamination. Further studies are therefore necessary to determine the real potential of detecting and quantify mycotoxins in wheat, other cereals or more complex matrixes (feeds or foods).

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