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Feed Analysis and Animal Nutrition: Electronic Nose as a Diagnostic Tool

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The electronic nose is an instrument that comprises an array of electronic chemical sensors and an appropriate pattern recognition system and capable of recognizing simple or complex volatile organic compounds' (VOCs) profiles associated to a product odour. The e-nose analysis of VOCs is of increasing interest as an analytical tool in many research areas, such as agricultural, food, pharmaceutical, biomedical, cosmetics, environmental, food, manufacturing, military. In the food industry, the electronic nose could represent a rapid and reliable tool for quality and safety assessment, freshness and shelf-life evaluation, authenticity assessment, foodstuff recognition, and process monitoring. This paper provides an overview of the applications of electronic nose in feed analysis and animal nutrition. Focus is placed on the applications as an analytical tool for quality control and management in the cereal and pet food industry. Further, this paper provides a critical outlook on the developments needed for transitioning of electronic nose use from research to industrial application in real contexts.

1. Introduction

A high quality and safe feed supply chain, based on feed analysis, is the foundation for the feed industry. In 2017, according to The European Feed Manufacturers' Federation (FEFAC), within the EU-28, approximately 480 million tons of feedstuffs have been consumed by livestock, and 156.7 million tons of compound feed were produced by EU feed industries, with cereals representing the 50% of the feed materials (FEFAC, 2017). Pet animals and the pet food industry contribute significantly to the economy and to human society. According to The European Pet Food Industry (FEDIAF), in 2017, 132 pet food producing companies and 200 production plants are present in Europe with a volume of 8.5 million tons and a turnover of 19.5 billion € as annual sales. Over the past 3 years, a 2 % average value of annual growth rate of the pet food industry has been registered (FEDIAF, 2017). The high level of feed and pet food production, the high-throughput testing demands of the feed/pet food industry and the regulatory enforcement have driven an increased need for quality control and, consequently, extremely high volumes of required analyses. To meet this demand, simplified rapid analytical methods that are non-destructive and cost-effective for use in high-volume routine analytical assays are needed. For the very challenging field of feed analysis, the potential applications of the electronic nose (e-nose) and the use of advanced mathematical procedures for signal processing represent the most promising "fit-to-purpose" tool to be routinely used in the industry for feed and pet food quality and safety evaluation purposes.
The main e-nose applications have been developed for quality monitoring of food and industrial processes and measurement of environmental pollution (Di Rosa et al., 2017; Guillot, 2016). The aim of this work is to provide an overview of the use of e-nose for feed analysis and animal nutrition, focusing on the applications as an analytical tool for quality control and management in the cereal and pet food industry.

2. The Electronic nose

The e-nose is an instrument that comprises an array of electronic chemical sensors, with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex volatile organic

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compounds' (VOCs) profiles associated to a product odour (Gardner and Bartlett, 1994). Volatile organic compounds can be used as quality markers and the VOCs' profile may represent a unique volatile fingerprint for monitoring feed quality and detecting changes during process and storage. The non-specific sensor response by an e-nose can be used for classification and prediction purposes. Moreover, e-nose analysis is rapid, user-friendly, precise, objective, non-destructive, and no or simple sample pre-treatment is required. Therefore e-nose represents a diagnostic tool useful for real time monitoring, control of products and industrial processes, and for decision-making in the area of product quality and safety.
The principal features and workflow of e-nose for feed analysis is reported in Figure 1.

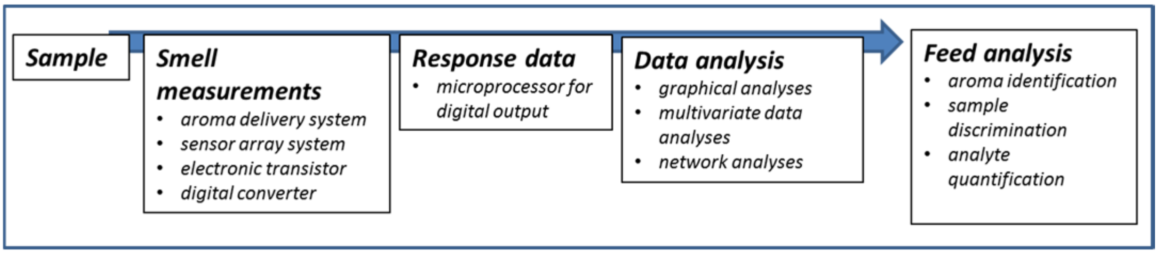

Figure 1: E-nose analytical workflow for feed analysis

Several types of sensors are employed in e-nose design. The most popular are MOS (Metal-oxide semiconductor), CP (Conducting polymer) and Piezoelectric crystal sensors, including BAW (Bulk acoustic wave) and SAW (Surface acoustic wave) (Di Rosa et al., 2017). The differences between sensors are linked with the nature of the technology and affect the response and recovery times, sensitivity, detection range, operating limitations, physical size, and inactivation by poisoning agents. The description of sensor characteristics and properties is not the aim of this paper. Interested readers on this topic are referred to specific reviews (Albert et al., 2000; Ampuero and Bosset, 2003; Wilson et al., 2009).
E-nose analysis generates a huge volume of data. In order to extract useful information from e-nose sensors' response, mathematical and statistical methods are required for data processing and for qualitative or quantitative analysis (aroma identification, sample classification, analyte quantification). Several pattern analysis techniques for e-nose data analysis can be used depending on the type of available output data acquired from the sensors and the type of information needed. The main categories are: graphical analyses, multivariate data analyses (principal component analysis - PCA, canonical discriminate analysis - CDA, cluster analysis – CA, linear discriminant analysis – LDA), and network analyses (artificial neural network - ANN, and radial basis function - RBF) (Wilson et al., 2009; Di Rosa et al., 2017).

3. E-nose for the cereal industry

Livestock is the most important outlet for produced cereals, large quantities being used by the feed industry and as feed directly on the farms of origin. In the EU-28, 27% of the cereal production is used by the feed industry and 34% on farm (FEFAC, 2017). In 2015, a world cereal production of 2,540 million tons has been reported. In 2017-2018, the total EU cereal production is forecast at 298 million tons. Rapid evaluation of cereal quality and safety represents a challenge for the feed industry helping to make rapid management decisions. As VOCs' pattern can be used as quality markers for cereal evaluation, e-nose may represent a rapid, low-cost, high-throughput analytical approach to be used at the industry level. The main application areas of e-nose for cereal analysis are reported in Table 1. In the field of mycotoxin detection, mainly commercial e-nose have been used (Table 2), while application of a non-commercial e-nose to detect mycotoxigenic fungi in contaminated grains has been reported (Eifler et al., 2011).
Among the most important safety risks associated to cereal consumption are mycotoxins (Pinotti et al., 2016). Mycotoxins are secondary metabolites of fungi, mainly *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., that have a great impact on human and animal health. Because of their worldwide occurrence and concern regarding human and animal diseases, aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxin A, T-2 and HT-2 toxins are the main contaminating mycotoxins to be analysed to ensure food and feed safety (Cheli et al., 2017). Appropriate maximum levels in foodstuffs and feedstuffs for these mycotoxins have been set worldwide and in the European Community (EU) (Cheli et al., 2014). It has been estimated that up to 25 % of the world's crops grown for foods and feeds may be contaminated with mycotoxins. This means that: 1) if the estimated world cereal production is about 2,500 million tons, there are potentially over 600 million tons of mycotoxin contaminated grains entering the food supply chain; 2) there is a need of rapid methods for mycotoxin detection. Fungal spoilage induces organoleptic deterioration and off flavour production associated

to mycotoxins formation (Sahgal et al., 2007). Recent experiments have been carried out to verify the ability of an e-nose to detect mycotoxin contamination (Table 2). These studies have been carried out with commercial e-noses. have been used to ones. I would like to know if there are studies with non-commercial electronic noses and if yes include some references.

Table 1: E-nose for cereal quality and safety evaluation (modified from Campagnoli and Dell'Orto, 2013)

Main topic	Application areas
Detection of VOCs as markers of potential grain spoilage	Fungal volatile compounds as indicators of food and feed spoilage Potential application of e-nose to the assessment of cereal quality Detection methods for moulds and mycotoxins in the food chain Detection of contaminants in bulk grain
Detection of mycotoxigenic fungi in contaminated grains	Evaluation of wheat contamination by *Fusarium poae* fungi VOCs in durum wheat during storage Detection and differentiation between mycotoxigenic and non-mycotoxigenic strains of *Fusarium* spp. Mycotoxins, ergosterol, and odorous volatile compounds in durum wheat during storage
Evaluation of mycotoxins in contaminated grains	Prediction of high and low fumonisin contamination in maize Detection and classification of aflatoxins in maize Recognition and classification of durum wheat naturally contaminated by deoxynivalenol
Early detection of insect odours	Detection of age and insect damage in wheat

Table 2: Application of e-nose for mycotoxin detection

Mycotoxin	Cereal	E-nose/sensor array	Multivariate pattern analysis technique	References
Aflatoxin, fumonisin	Maize, naturally contaminated	PEN3/10 MOS	DFA	Novacco et al., 2017
DON	Durum wheat, naturally contaminated	ISE Nose 2000/12 MOS	DFA	Lippolis et al., 2014
DON	Durum wheat, naturally contaminated	PEN2/10 MOS	PCA, CART	Campagnoli et al. 2011
Fumonisin	Maize, artificially contaminated	EOS[835]/6 MOX	PCA, PLS	Gobbi et al., 2011
Aflatoxins	Maize, naturally contaminated	PEN2/10 MOS	PCA, LDA	Cheli et al., 2009
OTA, citrinin	Durum wheat, naturally contaminated	FOX 3000/12 MOS	CORR	Abramson et al., 2005
DON	Durum wheat, naturally contaminated	PEN2/10 MOS	PCA, multiple regression	Tognon et al., 2005
DON, OTA	Barley, naturally contaminated	VCM 422/ 10 MOSFET,) sensors, 6 SnO_2, 1 Gascard CO_2	PCA, PLS	Olsson et al., 2002

DON: deoxynivalenol; OTA: ochratoxin; DFA: discriminant function analysis; PCA: principal component analysis; CART: classification and regression tree; PLS: partial least squares; LDA: linear discriminant analysis; CORR: correlation analysis.

E-nose was able to discriminate aflatoxin contaminated and non-contaminated maize with a classification accuracy of 100% (Cheli et al., 2009). E-nose analysis has been used to predict the fumonisin content of maize cultures for high and low contamination levels (Gobbi et al., 2011). E-nose analysis was able to discriminate wheat samples at contamination levels close to the DON maximum permitted limit set by the European Union for durum wheat (Campagnoli et al. 2011; Lippolis et al., 2014). The next challenge for e-nose use for mycotoxin contamination detection is represented by mycotoxin co-contamination that is more the rule than the exception (Cheli et al., 2017). Multi-mycotoxin studies reported a high incidence of 30–100% of analysed samples that were contaminated with two or more mycotoxins. E-nose analysis has been proposed to detect aflatoxins and fumonisin co-contamination in maize (Novacco et al., 2017). A classification

accuracy of 61% for co-contamination was achieved using discriminant function analysis, a performance still far away to meet the real needs of the cereal industry to detect mycotoxin co-contamination. However, it must be emphasized that the results referred to a limited number of samples. A larger datasets and better validation procedures may improve sample classification.
Overall results confirm that a properly validated e-nose analysis could be used as useful tool for high throughput screening of mycotoxin contamination. The major drawback of e-nose analysis is that it is capable of performing near real-time semi-quantitative prediction. Despite this limit, robust and suitable e-nose methods could be relevant and crucial to rapidly identify samples below or above the legal limits and, therefore, reduce the number of HPLC analyses (reference method) needed for evaluating the compliance of a product with the maximum levels established by Regulations.

4. E-nose for the pet food industry

Pet food palatability is one of the largest concerns in the pet food industry and a critical and competitive issue in determining the success in the market. The pet food industry continues to grow steadily as a result of new innovative products formulated to satisfy both pet's and owner's requirements. High quality ingredients, consumption rates and digestibility are critical points affecting pet development, health, welfare and the nutritional sustainability of pet food production. Dogs and cats use both smell and taste to select food. Therefore, the most nutritious pet food in the world is useless if it will not be eaten. Pet food palatability is related to the pet food formulation and the pet food sensory properties, such as flavour, aroma, texture shape, and particle size (Koppel, 2014). Microbial growth, oxidation, and the presence of undesirable compounds and contaminants represent risk factors responsible of changes flavours and loss of palatability. Besides ingredient composition, pet food palatability may be affected by the use of palatability enhancers and food processing. At the industry level, e-nose may represent a rapid, low-cost, high-throughput and online analytical approach in order to guarantee and standardize the quality and palatability of pet food. In literature, the applications of the e-nose in pet food analysis are very scarce (Table 3). This could be attributed to the need to tune either the hardware and/or software to a specific application, or because data are kept confidentially by the product developers (Cheli et al., 2017).

Table 3: Application of e-nose for pet food analysis

Samples	Applications	E-nose/sensor array	Multivariate pattern analysis technique	References
Commercial canned cat food	Pet food discrimination: - different composition - different brand	αFox 4000/18 MOS	DA	Éles et al., 2015
Cat palatants	Development of flavour profile	Heracles II/fast GC	PCA	Mohapatra et al., 2015
Commercial dry dog and cat pet food	Pet food discrimination: - dogs *vs* cat - complete *vs* dietetic - adult *vs* puppies	PEN2/10 MOS	PCA, DA	Battaglia et al., 2014
Commercial canned dog and cat food	Pet food discrimination: - pure animal *vs* animal and plant components	αFox 4000/18 MOS	DA	Éles et al., 2013
Off the shelf kibbles	Pet food discrimination: - different formulation - different manufacturing facilities	KRONOS e-Nose/	PCA	Oladipupo et al, 2011
Commercial dry dog food	Animal protein sources characterization in pet food	PEN2/10 MOS	PCA	Cheli et al., 2007

In literature, applications of e-nose analysis have been reported to discriminate dog and cat pet food, pet food of different brands, pet food with different ingredient composition, and pet food deferentially formulated for puppies or adult pets. (Éles et al., 2015; Battaglia et al., 2014; Éles et al., 2013; Oladipupo et al, 2011; Cheli et al., 2007). Results from studies on commercial pet food demonstrated that e-nose is able to discriminate, although not completely, dog and cat pet food according to their VOCs' profile (Battaglia et al., 2014; Éles et al., 2013). Interestingly, e-nose was able to discriminate complete pet food for puppies or adult dogs (Battaglia et al., 2014). Although more studies are needed to improve the discrimination performance of e-nose analysis, overall results confirm that pet food composition, brand and pet food process technology have a great influence on the VOCs' profile. Interestingly, results indicate that a combination of e-nose and e-tongue determined a better discrimination between samples (Éles et al., 2013). Another interesting field of application of e-nose is the characterisation of pet food palatants. E-nose has been successfully used to discriminate the flavour profile and identifications of key aromas and taste attributes of palatants used in the pet food industry (Mohapatra et al., 2015).

Overall results confirm that e-nose analysis could be used as useful tool for high throughput evaluation of the odour profile of pet food and palatants for quality control and research & development. E-nose analysis, combined with animal test, may be used to design and standardise highly palatable pet food. Once properly validated, e-nose analysis could replace animal preference test and chemical analysis to assess pet food palatability.

5. Conclusions

The e-nose technology represents a rapid and powerful tool for both quality control and management and research & development purposes in the feed and pet food industry.

In the field of cereal safety evaluation, e-nose has been mainly used to detect VOCs as biomarkers of grain spoilage by fungal and mycotoxin contamination. For single mycotoxin contamination, high discrimination accuracy between contaminated and non-contaminated grain has been reported. A robust and suitable e-nose method has been validated to discriminate wheat samples at DON contamination levels close to the maximum permitted limit set by the European Union. These data represent a relevant and crucial result with a high impact for the cereal industry. The rapid identification of samples below or above the legal limits for mycotoxins allows reducing the number of laborious, expensive, and time-consuming instrumental analyses needed to evaluate the compliance of a product with the levels established by Regulations. For mycotoxin co-contamination, further researches are needed. To date, in the field of mycotoxin co-contamination detection, the predictive accuracy of the e-nose models is still limited and unsuitable for industrial applications in a real context. From a technical perspective, contaminated samples misclassified as non-contaminated represents the worst outcome under in-field conditions in selecting samples that must undergo further accurate quantitative analysis.

Even for the pet food industry, e-nose could be used as useful tool for high throughput evaluation of the odour profile of pet food and palatants for quality control and research & development, although more studies are needed to improve the discrimination performance of e-nose analysis. Once properly validated, e-nose analysis could replace animal preference test and chemical analysis to assess quality and to design and standardise highly palatable pet food. Dogs and cats use both smell and taste to select food. Interestingly, a combination of e-nose and e-tongue determined a better discrimination between samples.

To conclude, for a complete transition of e-nose from research to industrial applications in real context, future work is needed on the sensor materials and data analysis, and a better understanding of the industrial needs related to quality control and monitoring of feed and pet food processing is required. Quality attributes of feed and pet food, in addition to aroma, include taste, colour, texture, size, and shape. Multi-sensor data fusion techniques represent the future challenge. E-nose could be integrated in an instrumental platform (electronic tongue, computer vision, …) for real time feed monitoring and management.

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