Sensors & Transducers, Vol. 201, Issue 6, June 2016, pp. 52-58

Sensors & Transducers

http://www.sensorsportal.com

Cereal Industry: e-Nose for Real Time and Online Quality and Safety Control and Management

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Received: 2 May 2016 /Accepted: 6 June 2016 /Published: 30 June 2016

Abstract: In the cereal industry, the on-site safety and quality of products and by-products need to be continuously monitored. At the industrial level, the main question is the choice of the best analytical method for a practical purpose enabling "decision-making" regarding the acceptance or rejection of a lot and the insurance of quality standards. Regular, economical, straightforward cereal tests with regard to a rapid and accurate diagnosis of food quality and safety are needed. The objective of this idea is to set up an electronic nose (e-nose) for the safety and quality evaluation of cereal products and by-products, focusing on mycotoxin contamination. The final goal is to evaluate the potential application of the e-nose technology as an on-line continuous monitoring and controlling tool in cereal processing, in particular wheat milling. E-nose could be integrated with other on-line analysis devices in a technological platform for monitoring and controlling food quality. Multi-sensor-devices and multi-sensor-data-fusion technology have a great potential value to the food industry to ensure that cereal products and by-products meet specifications according to their specific use. *Copyright © 2016 IFSA Publishing, S. L.*

Keywords: Cereals, Food safety, Mycotoxins, Electronic nose.

1. Introduction

During the last twenty years, the term food quality, in conformity with consumers' requirements and acceptance, has assumed a new and more complex meaning. Topics such as sensory attributes of a product (odour, colour and outer appearance), food safety (level of microbiological and toxicological contaminants), traceability and best manufacturing practices are of great importance to today's food industry for "total quality" evaluation. Many different sensing methodologies represent fast and precise potential tools for total quality evaluation, assurance and compliance with labelling and standards. The applications of the electronic nose (e-nose) are numerous in several areas related to the food industry, mainly in the field of quality and authenticity evaluation of fish, meat, milk, wine, coffee and tea.

Among the most important risks associated to cereals' consumption are mycotoxins. Globally, mycotoxins have a significant impact on human and animal health, economies and international trade [1]. A wide range of analytical methods for mycotoxin determination in food has been developed in recent years [2]. At the industry level, the adoption of a rapid, low-cost, high-throughput and on-line analytical approach is needed at all stages of cereal production and processing in order to guarantee the safety and quality of the production.

The idea of this paper is to set up an electronic nose (e-nose) for on-line continuous monitoring and controlling the safety and quality of cereal products and by-products focusing on mycotoxin contamination. To develop the idea and evaluate the potential application of e-nose as a potential tool for rapid management decision, a step by step procedure must be designed: knowledge of e-nose characteristics and applications in the cereal industry, proper selection of an appropriate e-nose system for the specific application, analysis of the cereal milling process to identify the optimal points for the e-nose analysis, analyse the critical points for the use of the e-nose in an integrated system for safety and quality evaluation.

2. Cereal Mycotoxin Contamination: the Global Context

2.1. Mycotoxin Occurrence in Cereals

Cereals and cereal by-products constitute the major part of the daily diet of human and animal populations. Among the most important risks associated to cereal consumption are mycotoxins. Mycotoxins are fungal secondary metabolites that have a great impact on human and animal health [3]. Latest estimates for world cereal production in 2015 and EU-28 production in 2014 are approximately 2,540 million tons and 323.3 mil tons, respectively [4, 5]. It has been estimated that up to 25 % of the world's crops grown for foods and feeds may be contaminated with mycotoxins [3]. This means that, 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. More than 300 mycotoxins are known. However, for practical consideration in food manufacturing, because of their worldwide occurrence and concern regarding human and animal diseases, the number is considerably less [6-7]. Aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxin A, T-2 and HT-2 toxins are the main contaminating mycotoxins in food.

Despite efforts to control fungal contamination, extensive mycotoxin contamination has been reported in both developing and developed countries. Specific reviews reporting the worldwide occurrence of mycotoxins in cereals are provided by several authors to whom the reader is directed [6, 8-10]. From the literature analysis, several critical issues emerge with a huge impact on the cereal industry: 1) considerable differences regarding the type and prevalence of mycotoxin contamination in different regions of the world have been reported; 2) seasonal and local weather conditions during critical plant growing stages (before, during flowering or in grain at maturity) influence mycotoxin contamination; 3) mycotoxin co-contamination is more the rule than the exception; 4) mycotoxins commonly occurring in cereal grains are not destroyed during most processing operations; 5) cereal processing affects mycotoxins distribution and concentration, concentrating mycotoxins into fractions that are commonly used as animal feed.

The impact of mycotoxins on human and animal health, the globalization of the trade in agricultural commodities and the lack of legislative harmonization have contributed significantly to the discussion about the awareness of mycotoxins entering the food supply chain. Mycotoxin regulations have been established in more than 100 countries, and the maximum acceptable limits vary greatly from country to country [11]. The European Union harmonized regulations for the maximum levels of mycotoxins in food and feed among its member nations [12].

2.2. Mycotoxins: the Analysis

Mycotoxins represent a major analytical challenge due to the wide range of chemical compounds and the wide variety of matrices in which they are found. Adequate sampling and analysis are necessary to make justified management decisions regarding what to do with lots that may be contaminated with mycotoxins. Sampling is the greatest source of error in quantifying mycotoxin contamination because of the difficulty in obtaining samples from large grain consignments and of the uneven distribution of mycotoxins within a commodity [13]. Sampling uncertainty dominates in final uncertainty result, and then the adoption of an on-line analysis may represent an interesting analytical approach to reduce this uncertainty. The Commission Regulation 401/2006/EC, laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs, provides precise details regarding the methods of sampling, acceptance parameters, criteria for sample preparation, analytical performance criteria of the methods of analysis that are used for the official controls, and criteria for reporting and interpretation of the results [14]. A wide range of analytical methods for mycotoxin determination in food and feed have been developed in recent years, such as high-performance liquid chromatography, gas chromatography, gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry (LC/MS/MS). LC-MS/MS instruments are becoming increasingly widespread for the determination of multiple classes of mycotoxins and of mycotoxin conjugates [15]. At the food industry level, the on-site safety of cereal and by-products needs to be continuously monitored. The main question is the choice of the best analytical method for a practical purpose enabling "decision-making" regarding the acceptance or rejection of a lot and the insurance of quality standards. Notwithstanding the availability of advanced methods, the great importance and need for mycotoxin quantification methods at the levels that are set by the European Commission for feedstuffs, the adoption of a rapid, low-cost, high-throughput analytical approach could represent a better option at the industry level, helping to make rapid management decisions [16].

3. 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 compounds' (VOCs) patterns associated to a product odour [17]. The conventional aroma analysis by gas chromatography–mass spectrometry (GC–MS) is too time-consuming, complex and labour-intensive for routine quality application. Compared to GC-MS, e-nose presents several advantages for manufacturers and processors (portable, ease to use, rapid response and low costs), which make it a powerful tool for screening analysis to address the needs for routine quality testing in the food industry. Therefore, the major differences between e-nose and standard analytical chemistry equipment are that e-nose 1) produces a qualitative output; 2) can often be easier to automate; 3) can be used in real-time analysis; 4) can be easily integrated in current production processes.

The main application areas of e-nose analysis to investigate the causes of cereal damage are reported in Table 1 (adapted from [18]).

Table 1. E-nose for cereal damage evaluation.

Main topic	Application area
Detection of VOCs as indicators of potential grain spoilage	Fungal volatile compounds as indicators of food and feed spoilage Potential application of EN to the assessment of cereal quality
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.*
Early detection of insect odours in grains	Detection of age and insect damage in wheat using an EN
Semi-quantitative/quantitative evaluation of mycotoxins in contaminated grains	Prediction of high and low fumonisin contaminations in maize Detection and classification of aflatoxins in maize Recognition and classification of durum wheat naturally contaminated by deoxynivalenol

Fungal spoilage induces nutritional losses, off-flavours, organoleptic deterioration often associated to mycotoxins formation. Researches have correlated fungal activity with the production of typical VOCs [19]. E-nose technique has been proposed as a new method for the detection of VOCs as markers of potential grain spoilage, detection and differentiation of mycotoxigenic strains of fungi in contaminated grains and semi-quantitative/quantitative evaluation of mycotoxin contamination [19-20]. This latter has been done using fungal VOCs as indicators of mycotoxin presence.

The use of e-nose as a screening tool for the presence of mycotoxins in food must take into accounts the maximum levels or guidance values established by legislation. Preliminary results are encouraging, showing that it is possible to use volatile compounds to predict whether the mycotoxin levels in grains are below or above maximum permitted levels (Fig. 1) [21-22].

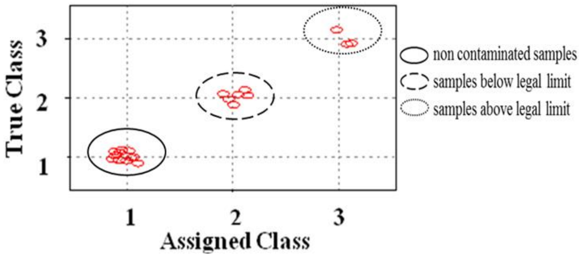

Fig. 1. E-nose for the recognition of durum wheat naturally contaminated by deoxynivalenol (CART-model performance plot) (adapted from [22]).

In addition to the issue of VOCs from fungal contamination, many characteristics that directly determine the effective quality and safety of a food are often described by its aroma. The e-nose is able to provide a global aroma fingerprint, which reflects the aroma complexity of a product. Thus, the evaluation of the "total quality" of food, which requires the simultaneous recognition, classification, and/or quantification of several parameters, could be achieved via a method based on the features and properties exhibited by e-nose. Consequently, e-nose could be applied for both research & development purposes and in-field applications (e.g., in food industry contexts and in production plants).

4. The Idea: e-Nose for Online Monitoring and Quality Control of Cereal Products, and Process

4.1. Set the e-Nose for the Specific Application

The suitability of an e-nose for a specific application is highly dependent on the required operating conditions (environment) of the sensor array and the composition pattern of the target VOCs. A proper selection of an appropriate e-nose system for a particular application must involve an evaluation on a case-by-case basis. E-nose selection for a particular application must necessarily include: assessments of the selectivity and sensitivity range of individual sensor arrays for particular target VOCs (i.e., related to mycotoxin contamination, target organoleptic properties of the products), the number of unnecessary (redundancy) sensors with similar sensitivities, as well as sensor accuracy, reproducibility, response speed, recovery rate, robustness, and overall performance. All these steps are common points of a validation procedure.

In order to configure an e-nose for mycotoxin detection, the different steps of the analytical workflow must be must be considered and set-up (Fig. 2).

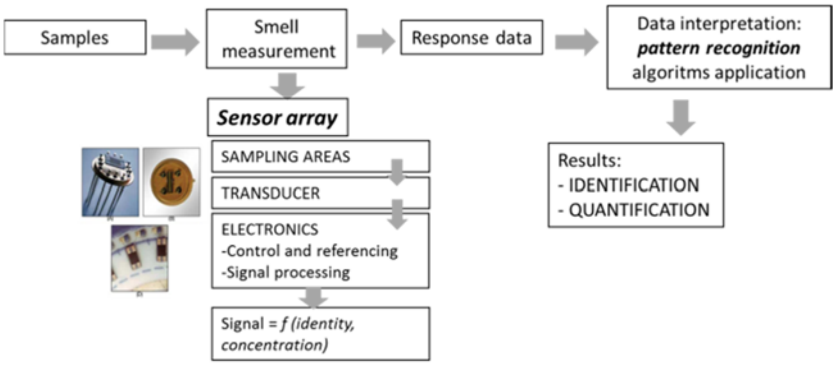

Fig. 2. E-nose analytical workflow.

The first step is the sample collection. Naturally wheat co-contaminated samples, analysed for mycotoxin contamination by instrumental analysis (LC/MS/MS), will be divided into two subsets. One of the two subsets, training set, will be used to calibrate the model, and the other one, validation set, will be used to verify the robustness of the established model.

The second step is the set-up of a protocol for the analysis of VOCs. The analyses will be performed on a PEN2 model EN operating with an EDU2 enrichment and desorption unit (EDU) from Airsense Analytics GmbH (Schwerin, Germany) and equipped with a HSS 32 headspace autosampler (Perichrom Sarl, Saulx-Les-Chartreux, France). The sensor array consists of ten metal-oxide-semiconductors (MOS), which characteristics are listed in Table 2.

Table 2. MOS Sensor Array of PEN 2.

No. in array	Sensor name	Description	Reference
1.	W1A – aromatic	Aromatic compound	Toluene, 10 mg/kg
2.	W5B - broadrange	Broad range sensitivity reacts to nityrogen oxides and ozone very sensitive with negative signal	NO_2, 10 mg/kg
3.	W3A - aromatic	Ammonia, used as sensor for aromatic compounds	Benzene, 10 mg/kg
4.	W6B - hydrogen	Mainly hydrogen, selective (breath gases)	H_2, 100 mg/kg
5.	W5A – arom-aliph	Alkanes, aromatic compounds, less polar compounds	Propane, 1 mg/kg
6.	W1B – broad - methane	Sensitive to methane (environment) ca. 10 mg/kg. Broad range, similar to No. 8	CH_4, 100 mg/kg
7.	7	W1C-sulphur-organic	Reacts on sulphur compounds H_2S 0.1 mg/kg. Sensitive to many terpenes and sulphur organic compounds, which are important for smell, limonene, pyrazine.
8.	W2B- broad - alcohol	Detects alcohols, partially aromatic compounds, broad range.	CO, 100 mg/kg
9.	W2C- sulphur-chlor	Aromatics compounds, sulphur organic compounds	H_2S, 1mg/kg
10.	W3B- methane-aliph	Reacts on high concentration > 100 mg/kg, sometimes very selective (methane).	H_4, 10 mg/kg

Samples will be analysed either with thermal desorption pre-treatments or without thermal desorption. All parameters involved in the headspace sampling and analysis will be optimized to obtain the best compromise between sensor responses and measurement time. The ratio G/G0 (where G and G0 are the resistance of a sensor in a detecting gas and in clean air, respectively) will be recorded by the e-nose dedicated software. For experimental purposes, three aliquots of each sample will be singularly analysed, and the mean value of the sensor signals from each aliquot will be calculated and recorded as a single odour profile.

The last step is the feature extraction and data processing. Pattern recognition systems (principal component analysis - PCA; linear discriminant analysis - LDA) will be employed to select variables and build a model to improve the sample discrimination analysis and create models to be used as quality control process tools.

Results from the e-nose equipped with MOS sensors will allow to identify the best analytical protocol for mycotoxin analysis in wheat, to evaluate the capability of e-nose to classify wheat samples into different clusters based on mycotoxin content: absence/presence below or above legislative limits. Regarding this topic, an important final consideration for designing e-nose systems as a rapid screening tool for food industrial applications is the incidence, frequency and acceptable rate of false classifications. Moreover, results may give crucial information for the development of on-line e-nose devices involving a reduction in sensor number (relative to larger bench-top laboratory instrument versions) and identifying specific sensor types in the array to optimize the performance for specific applications.

Besides mycotoxin analysis, the set up and validation protocol could be used for enhancing the performance of the sensor system for a "total quality evaluation". New ways to improve e-nose performance using better or more target-specific sensors, pattern-recognition algorithms, data analysis methods, will significantly amplify the range of applications of e-noses in the food industry.

4.2. Critical Points in the Cereal Milling Process for Online e-Nose Analysis

Industrial milling technology is a very complex process and presents several key processing steps that differently influence mycotoxin repartitioning in cereal by-products. Published data confirm that milling reduces mycotoxin concentration in fractions used for human consumption, but concentrates mycotoxins into fractions commonly used as animal feed [23]. However, these fractions may represent promising novel food ingredients with a high value for human nutrition, too. Physical processes carried out before milling (such as sorting, cleaning, debranning) are interesting and efficient methods to reduce the grain mycotoxin content before milling. These processes may be even more efficient than conventional milling. A high variability in mycotoxin repartitioning was reported and sometimes inconsistent results were reported. This may be mainly due to the type of mycotoxins, the level and extent of fungal contamination, and an omission of the description of the complexity of milling technology [23].

Therefore, food process control needs real-time monitoring at critical processing points. Very schematically, the chain of the cereal milling process includes three main steps: receiving and storage of grains, production (milling process) and storage of products and by-products (Fig. 2). The dry milling process of wheat is a gradual reduction process by which wheat is ground into flour or semolina, including several steps, such as cleaning and sorting, debranning and milling. At the industrial level, several on-line technological solutions for rapid and non-destructive analysis and quality control of the grain before and after milling are available, such as optical sorters, near infrared (NIR)-based analysis technology, on-line colour, contrast and ash control of cereal products.

The e-nose could be used as an on-line sensing tool at different point (Fig. 3): 1) receiving and storing of grain to control the safety aspect (i.e., mycotoxin contamination); 2) at the end of the milling process to control the quality and safety of the finished product (i.e., mycotoxin contamination, organoleptic characteristics, etc.); 3) after storing before products are packaged or delivered to ensure that they meet specifications according to the specific use. E-nose data may be continuously calculated, transmitted and integrated in the process control system.

4.3. e-Nose in an Integrated System for Total Quality Evaluation

In order to achieve a "total quality evaluation" of cereal products and by-products, e-nose data should be integrated with data from several on-line technological solutions for rapid and non-destructive analysis already are available (like those mentioned before, i.e., optical sorters, NIR-based analysis technology, etc.) to create a technological platform for food process monitoring. Multi-sensor data fusion is a technology able to combine information from several sources in order to improve the monitoring process performance (Fig. 4).

Low-level data fusion (direct integration of the raw data of various sensors) and intermediate-level data fusion (data fusion after the feature extraction process to keep enough raw information and eliminate redundant information) will be evaluated for classification efficacy in food quality evaluation. However, the final goal is to create a high-level fusion, namely decision-making fusion, able to analyse the features from each analytical system first and then to associate these features to produce a fused result. For intelligent loops, all the data must be transmitted to the process control system for a continuous quality assurance. Alarm messages must be issued in the case of drift-away from the target value to advice the operators.

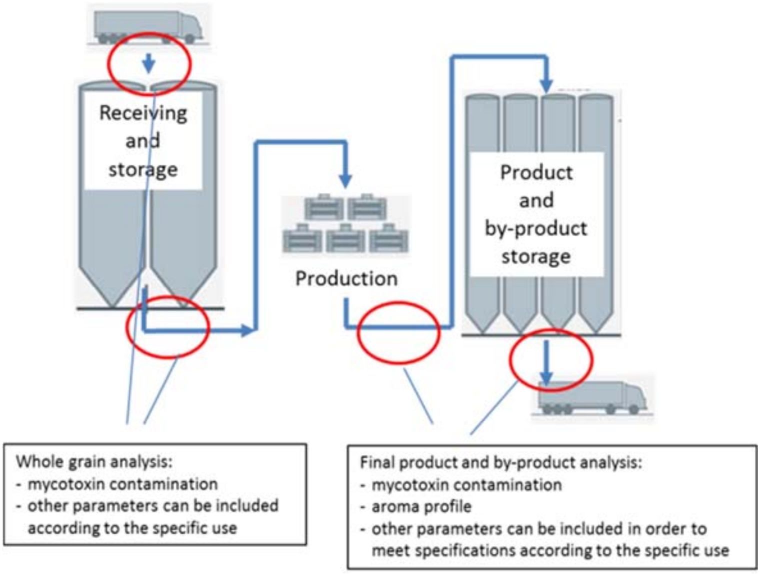

Fig. 3. Schematic representation of the chain of the cereal milling process and the possible e-nose optimal points for product and process "total quality" evaluation [24].

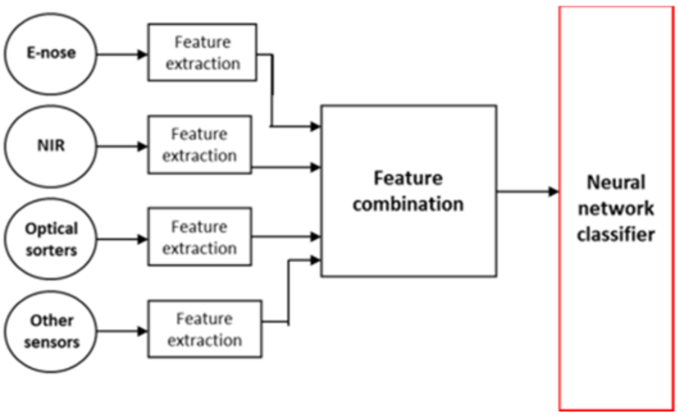

Fig. 4. Multi-data fusion for characterization of food safety and quality.

5. Conclusions

E-nose represents a powerful tool for safety and quality evaluation in cereal processing. Despite the advantages of e-nose analysis, there are still few applications of e-nose adopted in industry. This could be attributed to the need to tune either the software and/or hardware to a specific application. Therefore, future work is needed on the materials' side, on the data analysis side and on the industrial side (Table 3).

Although rapid methods for *on-site* mycotoxin measurements are available, the time and effort that are required to obtain a representative sample may still represent a limit for the rapid screening of mycotoxin contamination. Recently, evidence for a significant correlation of concentrations of deoxynivalenol in grain dust and by-products of grain cleaning with concentrations in whole grains has been given [25]. Therefore, the sampling and analysis of dust and by-products of cereal grain cleaning may represent an opportunity to improve *on-site* rapid mycotoxin measurements and a promising tool for control and mitigate the mycotoxins problem at the industrial level.

Table 3. E-nose for the cereal industry: future needs.

Item	Research needs
Sensors	New material for better selectivity, design and development of sensors that can be used reliably over long temporal horizons
Data analysis	Better modelling and correlation between chemical markers and the sensor response Neural network analysis Development of data fusion analysis for the process control system for a continuous quality assurance
Cereal industry	Better understanding of the industrial needs related to quality control and monitoring of food processing

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