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Rapid Methods as Analytical Tools for Food and Feed Contaminant Evaluation: Methodological Implications for Mycotoxin Analysis in Cereals

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1. Introduction

Over the past years, food quality is perceived to have improved and food safety has become an important food quality attribute (Röhr et al., 2005). This implies that all aspects of food production and therefore of the feed supply chain must be considered to ensure the safety of human food (Pinotti & Dell'Orto, 2011).

As a result, public authorities and regulatory agencies are pushing producers, manufacturers, and researchers to pay serious attention to food and feed production processes and to develop comprehensive quality policies and management systems to improve food safety and try to enhance consumer information to regain consumers trust in food.

From this point of view, the knowledge and control of the level and distribution of contaminants and undesirable substances in food and feed are become a worldwide topic of interest due to the high economic and sanitary impact on human/animal health. Since it is impossible to fully eliminate the presence of undesirable substances and contaminants, an adequate surveillance and frequent checks are fundamental to assure quality and safety of raw materials destined for direct consumption or industrial processes.

To guarantee food safety, the availability and the need for confirmatory methods of analysis with high sensitivity/accuracy to meet the regulatory requirements remain critical. However, the traditional methods have some typical drawbacks which include: high costs of implementation, long time of analysis and low samples throughput, and the need for high qualified manpower (Tang et al., 2009). The availability of fast, reliable and simple to use detecting tools for food feed products is therefore a target both for the safeguard of customer's health and production improvement (Tang et al., 2009) and it is undoubtedly one of the main challenges and an imperative for a modern feed and food industry.

In recent years, a number of cost-effective and fit-for-purpose approaches have been proposed to determine the effectiveness of the safety measures and to achieve logistical and operational targets. From this point of view, rapid analytical methods would keep commodities and products moving rapidly through the industrial processes, saving time and requiring less technical training. Analytical approaches that provide qualitative or semi-quantitative results for many chemical and microbiological applications are available and would reduce costs by operating a selection of samples to be submitted to more expensive, sensitive and specific analyses and can be recommended for use in sample screening. Among these, a group of rapid methods comprises some approach miming human/animal senses, for instance electronic nose. In many cases, these devices offer a particular kind of information, pointing on a general description of samples rather than providing a set of specific “discontinuous” analytical responses. This further aspect could result useful, under specific conditions, to give an evaluation regarding the “total quality” value of the matrices with a single analysis.

The aim of this chapter will be to evaluate the potentiality offered by rapid analytical approaches to food and feed evaluation, focusing on contaminants and undesirable substances. A critical overview, highlighting characteristics and applications of these techniques, will be offered with examples pointed on specific matrices and contaminants, cereals and mycotoxins, respectively.

2. Food and feed contaminants: Mycotoxins

Cereals are still by far the world's most important sources of food, both for direct human consumption and indirectly, as inputs to livestock production. FAO's latest forecast for world cereal production in 2011 stands at nearly 2 313 million tones, 3.3 percent higher than in 2010 (FAO, 2011). For the feed sector, cereals represent the main components of industrial feeds, which estimated production, worldwide, is more than 717 million tons (Best, 2011). These volumes make extremely complex the issue of the control and evaluation of quality and safety features and extremely high the amount of analysis that must be performed to meet the regulatory requirements or to give added value to products intended for human and animal consumption. In terms of food safety, cereals represent very heterogeneous materials characterized by a large set of undesirable substances and contaminants. Among the most important risks associated to cereals' consumption are mycotoxins (Codex Alimentarius, 1991).

Mycotoxins are metabolites of fungi capable of having acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic, and oestrogenic effects in man and animals (D'Mello et al., 1999; Wild & Gong, 2010). Since the discovery of aflatoxins in 1960 and subsequent recognition that mycotoxins are of significant health concern to both humans and animals, mycotoxins have received considerable attention as biotoxins in the food chain. Extensive mycotoxin contamination has been reported to occur in both developing and developed countries. It has been estimated that up to 25% of the world's crops grown for feed and food may be contaminated with mycotoxins (Fink-Gremmels, 1999; Hussein & Brasel, 2001). These data are in line with those reported by the Rapid Alert System for Food and Feed in the European Union (RASFF, 2009), for which of total 3 322 information notifications of possible risks to human health, 669 were related to mycotoxins. This also means that, if the estimated

world production is about 2 300 million tonnes (2011), there are potentially about 500 million tonnes of mycotoxin contaminated grains entering the feed and food supply chain. Furthermore, according to the possible carry-over of mycotoxins, feed contamination can represent also a hazard for the safety of food of animal origin and can contribute to mycotoxin intake in human population (Monaci & Palmisano, 2004; Jorgensen, 2005). In this context, one of the latest surveys (Taylor-Pickard, 2009) confirms that feedstuffs are typically contaminated with more than one toxin, which may have a cumulative effect in terms of toxicity in the animals. This places a number of economic and food safety risks for growers, cereal food business operators and food and feed manufacturers. The risks of contamination are greater when raw materials are not traceable or derive from countries where adequate monitoring infrastructures are not in place (Pinotti et al., 2005;). In this field, the geographic origin of food and feed material is also important (Pinotti & Dell'Orto, 2011). Although it is known that mycotoxins are ubiquitous and not just limited to humid and hot countries, where the climate is more favourable to microbial and fungal contamination, it has been reported that some toxins can occur more frequently than other according to the producing area of the food/feed material. Thus zeralenone, fumonisin and aflatoxin were the most widespread toxins found in Asian commodities. By contrast, zeralenone and deoxynivalenol were the most prevalent toxins in continental Europe samples, even after adjusting for the seasonality of contamination for these different toxins (Taylor-Pickard, 2009). By-products typically contain higher levels of toxins' contamination compared to whole raw materials. From a safety perspective, it is well documented that milling and thermal processing such as baking, extrusion cooking and roasting are treatments that may affect redistribution, stability, change and removal of mycotoxins in the processed food (Brera et al., 2006; Bullerman & Bianchini, 2007; Castells et al., 2008; Cheli et al., 2010). Therefore, controls are needed at all stages of cereal production and processing in order to guarantee the quality and safety of the production.

The knowledge and control of the level and distribution of mycotoxins in food and feed are a worldwide objective of producers, manufacturers, regulatory agencies and researchers due to the high economic and sanitary impact on food and feed safety and human/animal health. As stated before, since it is impossible to fully eliminate the presence of undesirable substances and contaminants, maximum concentrations should be set at a strict level which is reasonably achievable considering the risk related to the consumption of the food and, consequently, an adequate surveillance and frequent checks are fundamental to assure quality and safety of raw materials destined for direct consumption or industrial processes. Communities fixed maximum levels for mycotoxins in foodstuffs through the Commission Regulation (EC) No 1881/2006 of 19 December 2006 and Commission Regulation (EC) 1126/2007 of 28 September 2007. In the field of animal nutrition, specific indications on mycotoxins and other undesirable substances in animal feed are considered in the Commission Directive 2003/100/EC of 31 October 2003 and in the Commission Recommendation 2006/576/EC of 17 August 2006.

3. Contaminated food and feed as analytical matrices. Approach to error reduction during sampling and analytical procedures

Ingredients for human foods as for animal feeds are typically very heterogeneous and complex matrices to be analyzed. On the other hand, food and feed contamination can be

heterogeneous as well, including biological, chemical and physical contaminants. The biological contamination, comprising microorganism, natural occurring toxins (i.e. mycotoxins from fungi, phycotoxins from algae, toxins from cyanobacteria, histamine, vegetal alkaloids, etc.), and chemical contamination (i.e. agrochemicals as pesticides, plant growth regulators, veterinary drugs, and environmental contaminants as metals, dioxins, BCBs, etc.) get more concern for food and feed safety (Tang et al., 2009). When contaminants and undesirable substances have to be detected or quantified with reasonable confidence, a further critical aspect must be considered, such as their distribution, within a lot to be analyzed. This can be very different due to the characteristics of both food/feed matrices and undesirable molecules themselves. Usually contaminants are divided into two groups, substances uniformly distributed (pesticides, additives, heavy metals, PCBs, dioxins, medicine residues, etc) and non uniformly distributed (natural toxins, GMO, salmonellae, etc.). The type of distribution of contaminants in food and feed has major implications for attempting to precisely and accurately measure the level of contamination in a commodity bulk that is fundamental for products intended for food/feed uses in order to respect the final purposes, i.e. fixed maximum tolerable levels or other operational targets for food/feed industry. Once again a good example is provided by mould and mycotoxin distribution in food and feed commodities. It is well known that mycotoxin contamination is heterogeneously distributed in raw materials (Whitaker, 2004; Larsen et al., 2004). Bulk cereal moisture usually facilitates the development of localized clumps particularly rich in moulded kernels. These small percentages of extremely contaminated portions ("hot spots") are randomly distributed in a lot (average value usually registered about 0.1%) (Johansson et al., 2000a). This condition can lead to an underestimation of the real level of mycotoxin if a too small sample size without contaminated particles is analysed or, instead, to an overestimation of the true level in the case of a too small sample size featuring or more contaminated particles are analysed. Accordingly, when a quantification for a specific contaminant has to be performed in a specific food matrix, all the above mentioned aspects give a fundamental contribute to sampling variability, uncertainty of measurements and finally, to analytical results (Cheli et., 2007a). For these reasons, an analytical methodology to really be considered "fit-for-purpose" should be chosen taking into account not only the sensitivity / specificity, precision and accuracy of the measurement technique adopted, but also its compatibility with an adequate sampling method. In fact, under certain circumstances, as in the case of above described complex, coarse matrices and/or contaminants characterized by the tendency to heterogeneous distribution into the matrix, it appears intuitive that the sampling error could account for an important part of the total error of the final result. On the other hand this topic reveals further interesting implications. If is concrete the hypothesis that, in a specific condition, sampling uncertainty dominates in the uncertainty of the final result, then the choice of an expensive and effective analytical method could result an inefficient strategy. Otherwise, the adoption of a rapid, low cost and high sample throughput analytical approach able to test a high number of samples can represent a better option (Fearn, 2011). From this point of view some statistical approaches can represent helpful tools not only for results' analysis and final data interpretations but also to estimate the importance of the sampling error and in general to estimate the usefulness of a specific analytical application (French, 1989).

As a consequence, the definition of the concept of sampling procedure (also defined "Sampling plan"), and of sampling strategy, as a function of the final target of analysis, and, when possible, the selection of the opportune analytical technique, including rapid methods, represent topics that deserve further in-depth examination in order to achieve the optimization and the fitness of purpose of an analytical approach for contaminant evaluation in food and feed.

3.1 Plan a sampling procedure for mycotoxins

A sampling plan for mycotoxins may be defined as a "test procedure combined with a sample acceptance limit" (Johansson et al., 2000b). A sampling procedure is a multistage process and consists of a sampling phase and an analytical phase. The analytical phase can be further splitted into sample preparation and instrumental analysis (Whitaker, 2006). All the phases are associated to a variability which can impair the reliability of the final result. Each phase of a sampling plan is associated to a specific level of uncertainty and therefore, as mentioned above, in no circumstance is it possible to obtain a quantitative value for the contamination associated with 100% certainty (Whitaker, 2006). It is intuitive that each step of a sampling protocol specifically contributes to the final uncertainty of the procedure. The total variance of a specific sampling plan (TV) may be expressed by using statistic variance as a measure of variability and may be described as the sum of sampling variance (SV), and analytical variance (AV) as follows (1):

$$TV=SV+ AV \quad (1)$$

(in which AV reassumes the sum of sample preparation variance (SPV) plus instrumental analysis variance (IV)). TV and variance distribution in the different steps of the sampling protocol give indications on the sampling plan efficiency and are also able to compare effectiveness of different sampling plans to the final purpose (Cheli et al., 2009a).

The contribution from SV has often been underestimate, though it is accountable for the largest source of variation associated to the quality of the final analytical result (Whitaker, 2003, Cheli et al., 2009a). There appears to be more substantial literature on food than feed (Cheli et al., 2009a).

Due to the frequently uneven contaminant and undesirable substance distribution in solid samples, such as grains and other alimentary commodities, raw material and matrices, obtaining a representative sample is a way of minimizing false results and increases the chances of accurate determination of mycotoxins in a batch or lot. When designing a specific sampling plan, all critical points have to be considered in order to reduce SV and increase the reliability of the final sample, such as collection of a sufficiently large number/size of incremental samples, choice of the sampling points, aggregate sample size properties, homogeneity of sample components in terms of size and specific weight. All these parameters must specifically consider the type of product and mycotoxin level of contamination. For mycotoxins, it becomes even more important than usual to consider the contribution of SV to the uncertainty of any measurement, and there are implications for the type of measurement technology that may be judged fit for purpose. The contribution of SV, SPV and IV to TV has been evaluated and quantified in several products (Table 1). In this context, quantitative data are available for foodstuffs, but are still lacking for the majority of feedstuffs.

Matrix, mycotoxin and test procedure	SV, %TV	SPV, %TV	IV, %TV	References
Shelled corn, 0.91 kg sample, Romer mill, 50 g subsample, 1 aliquot analysed, aflatoxin 20 ng/g	75.6	15.9	8.5	Whitaker, 2006
Shelled corn, 4.54 kg sample, Romer mill, 100 g subsample, 2 aliquots analysed, aflatoxin 20 ng/g	55.21	29.1	15.7	Whitaker, 2006
Shelled corn, 1.13 kg sample, Romer mill, 50 g subsample, 1 aliquot analysed, aflatoxin 20 ng/g	77.8	20.5	1.7	Johansson <i>et al.</i> , 2000c
Wheat, 0.454 kg sample, Romer mill, 25 g subsample, 1 aliquot analysed, Deoxynivalenol ppm	22	56	22	Whitaker <i>et al.</i> , 2002
Shelled corn, 5 kg sample, Romer mill, 100 g subsample, 1 aliquot analysed, aflatoxins 20 ng/g	59.8	34.5	5.7	Johansson <i>et al.</i> , 2000c
Peanut, 2.27 kg sample, 100 g subsample, aflatoxin 100 ppb	92.7	7.2	0.1	Whitaker <i>et al.</i> , 1994
Shelled corn, kg sample, 25g subsample, 1 aliquot analysed, fumonisin 2 mg/kg	61	18.2	20.8	Whitaker <i>et al.</i> , 1998

Table 1. Distribution of variability associated to each sampling step: sampling (SV), sample preparation (SPV) and instrumental analysis (IV) (modified from Cheli *et al.*, 2009a).

The methods of sampling and analysis for the official control of the levels of mycotoxins, are reported in Commission Regulation (EC) No 401/2006 of 23 February 2006 and Commission Regulation (EC) No 152/2009 of 27 January 2009. These regulations provide different sampling plans according to the type of food and feed products, respectively. However, screening, monitoring, controlling, exposure studies or targeted purposes may require specific sampling and analytical approaches (Miraglia *et al.*, 2005).

3.2 Toward optimization of sampling and analysis procedures

Some aspects related to sampling plan evaluation and the establishment of a decision strategy are more detailed by Fearn *et al.* (2002) in an interesting paper in which the authors describe a possible approach to the systematic optimization of the different phases during the entire sampling procedure. Later on, this approach enables an economic evaluation of

the entire process, and, as a consequence, an objective comparison among different plans applicable to the same situation. Cost can be in fact defined as the measurement unit to take the optimal decision if it is considered that the optimal decision represents the choice of the most economic from different plans when quality of results are comparable. In a sampling procedure, total cost can be defined as the analytical cost plus the potential losses incurred in using the result.

To plan a sampling procedure, the analytical method, numbers of replicates samples, numbers of replicate measurements per samples and the sampling technique have to be selected. Thus, a systematic approach is first to optimize numbers of replicate samples and analyses separately for each combination of sampling technique and analytical methods. Then the optimised total costs of different methods may be compared.

As described in 3.1 paragraph, the uncertainty of the measurement can be expressed in terms of total measurement variance, calculated as the sum of sampling and analytical variance. Considering a measurement process in which n samples are taken and m replicate analyses per sample made, the uncertainty of the measurement is dependent on the number of samples and replicate analyses. Increasing the number of samples and/or analyses will reduce the uncertainty but will increase cost to obtaining the measurement. For a given cost, different allocations of resources between sampling and analysis may give different variances. As a consequence, for a fixed cost, a balance between sampling and analysis may be found with the aim to reach the best economic purpose and the minimum total measurement variance (besides usually there are few sampling or analytical methods available for a given problem so the choice can be simplified).

Thus, the total variance of the sampling plan can be more completely described as in (2)

$$TV^2=(SV^2/n)+(AV^2/mn) \quad (2)$$

where n is the total number of samples taken and m is the number of analyses carried out on each sample; while the total cost of obtaining the measurement (cost of the entire sampling plan) (TC) including sampling (SC) and analysis cost (AC), can be defined as in (3)

$$TC=nSC+mnA \quad (3)$$

Either fixing the cost TC and minimizing the variance TV^2 or vice versa, the optimal number of replicate analyses can be shown to be (4)

$$m_{opt}=(SV/AV)\cdot\sqrt{(SC/AC)} \quad (4)$$

The value of m will need to be rounded to the nearest whole number. New rounded value for m give important information. If m does not seem sensible, this may indicate that the sampling and analytical methods are badly matched. Large values of m_{opt} will result if the analytical variance is large compared with sampling variance or if the sampling cost is large compared with the analytical cost. Then it may be better considering more precise analyses or less expensive and less precise sampling procedures to get a better balance. Of course not all choices can be permitted and each operational situations allow a specific range of possibilities, so some compromise value of m will need to be chosen. It will rarely be a good idea to make more than 4 or 5 replicate measurements on a sample. Values of much less

than one for m_{opt} will occur if the sampling variance or analytical cost dominate. Again may be useful to consider alternative analytical procedures that are less precise and therefore less costly.

A practical example can be done. Starting from the assumption that the standard deviation and the cost for single sample of an analytical method are usually known and that frequently when a sampling methodology is consolidated the relative standard deviation and cost can be inferred, we can suppose the sampling has a SD=0.8 with a cost of 21.00 Euros, while the analysis has a SD=0.6 and a cost for single sample of 4.00 Euros. m_{opt} will be calculated as (5)

$$m_{opt} = (0.6/0.8) \cdot \sqrt{(21.00/4.00)} = 1.72 \quad (5)$$

so m_{opt} will be approximate to 2. Then each sample will cost 29.00 Euros (21.00+2*4.00) and results associated to a SD=0.91.

After having obtained cost and SD of result, the next step is to find the optimal level of sampling replications n , balancing measurement costs against possible losses. When choosing a value for n , then each optimized method can be compared with the other candidate methods. If the optimal n is less than one in situations where an m of greater than one has been used it may be reasonable trying smaller values of m .

When optimising each method separately, then they can be compared by comparing the total costs. In the absence of other operational or technical considerations the least cost option will be chosen.

As general consideration the use of a decision strategy like those described allows a rational approach to the problem of choosing analytical methods, a sampling scheme and how to mach efficiently these two phases of the sampling procedure. Under certain circumstances, there is no doubt that some parameters may be difficult to quantify. Probably for instance, the most problematic of the inputs will usually be the losses arising from measurement errors. In situations where the potential losses are very large, it may be necessary to take account of a nonlinear utility for money. Despite these aspects, it can be state that is still possible to get useful results from this approach.

4. Rapid methods for mycotoxin analysis

The use of so called "Rapid Methods" is highly relevant for improving the knowledge on the presence and distribution of mycotoxins in food and feed and for creating a reliable database (Stroka et al., 2004). These low cost, simple, rapid and reliable methods may be applied in laboratory and non-laboratory environment and combine effective sampling with analysis of a large number of samples for a screening approach. As a general rule, rapid methods that provide qualitative or semi-quantitative results are recommended in sample screening. An analytical method is usually referred to as "rapid" when it requires, at most, a few minutes to obtain a result (van Amerongen et al., 2007). Currently, there are three main tendencies to develop rapid methods for mycotoxin analysis in order to reduce the quantity of assays and, therefore, to shorten time and to lower costs for feed and food quality control: 1) improvement of speed, user-friendliness, reliability, non-destructiveness, 2) use in a non-laboratory environment, 3) simultaneous determination of multiple mycotoxins (Maragos,

2004). In recent years, a number of rapid, cost-effective and fit-for-purpose approaches have been proposed to determine the effectiveness of the safety measures, to determine legal compliance, to achieve logistical and operational targets, to keep commodities and products moving rapidly through marketing channels, to save time and investments in complex instruments. Some are advanced enough for field studies and have already reached the stage of commercialization, some are at a transition phase between research and application to analysis of food/feed samples, other still have to face the challenge of validation by multiple laboratories. A list of the emerging rapid methods for mycotoxin analysis is reported in Table 2.

Methods	Advantages	Disadvantages	References
LFD (lateral flow device)	Rapid No expensive equipment Easy to use	Semi-quantitative Validation required for each matrix	Maragos, 2004; Zeng et al., 2006; Goryacheva et al., 2007.
FPI (fluorescence polarization immunoassay)	High sensitivity Low matrix interference	Not usable for simultaneous detection of several individual mycotoxins	Maragos, 2004; Goryacheva et al., 2007.
CE (capillary electrophoresis)	High sensitivity Non polluting technology Possible simultaneous multi-component analysis	Expensive equipment Expensive Clean-up may be required	Maragos, 2004; Maragos & Appel, 2007.
SPR (surface plasmon resonance)	Rapid No clean up	Cross reactivity	Tudos et al., 2003; Van der Gaag et al., 2003; Maragos, 2004.
MIP (molecularly imprinted polymers)	Low cost Stable Reusable	Poor selectivity	Maragos, 2004; Logrieco et al., 2005; Krska & Welzig, 2006.
IR spectroscopy (NIR, FR-NIR)	Rapid Non destructive measurements No clean up Easy to use	Expensive equipment Calibration model must be validated Good for classification	Kos et al., 2002, 2003; Petterson & Aberg, 2003; Berardo et al., 2005; De Girolamo et al., 2009.
EN (electronic nose)	Rapid Non destructive measurements No clean up	Calibration model must be validated Good for classification	Keshri & Magan, 2000; Olsson et al., 2002; Presicce et al., 2006; Cheli et al., 2009b; Campagnoli et al., 2011.

Table 2. Examples of emerging rapid methods for mycotoxin analysis.

Emerging technologies and their potential application in rapid mycotoxin detection have been recently reviewed (Maragos, 2004; Krska & Welzig, 2006; Zeng et al., 2006; Goryacheva et al., 2007; Cheli et al., 2008; Maragos & Busnam, 2010). The most known rapid screening methods for mycotoxin detection, especially for the screening of raw materials, are antibody-based methods, ELISA test. The ELISA methods have been commercially available since many years and are extensively used as rapid screening methods. Kits are available in quantitative, semi-quantitative or qualitative formats (Zeng et al., 2006). These methods are easy to use, fast and suitable for testing mycotoxin in the field too. Within the concept of flexible out of laboratory testing, non instrumental (visual) membrane based immunoassays (dipstick, lateral flow and flow-through tests) have been developed and are commercially available for several mycotoxins and matrices. The main advantages of non instrumental ELISA methods are field portability, not requirement of any specialized equipment and simple sample preparation procedures, while the main disadvantages are subjective interpretation, lower sensitivity and higher cost/test compared with instrumental ELISA methods (Zeng et al., 2006; Goryacheva et al., 2007). Although immunochemical methods have become one of the most useful tools for mycotoxin rapid screening, the price for simplification may be usually lower sensitivity. The main problems with antibody-based methods are related to the characteristics of the antibody, test specificity (cross-reactivity), matrix interference and interpretation of the result, if the method is semi-quantitative, when the mycotoxin concentration is close to the method cut-off level. Still insufficient validation studies of ELISA methods for all commodities limit their use to those matrices for which they were validated.

Apart from ELISA, the more recent and best candidates as mycotoxin analytical methods for further developments in terms of rapid methods, multi-mycotoxin assays, easy to use and to be validated by multiple laboratories are capillary electrophoresis (CE), fluorescence polarization immunoassay (FPI) and surface plasmon resonance (SPR). CE methods are laboratory-based methods because of the size and required automation of the instrumentation, while FPI and SPR methods may be much more portable and therefore may be used outside the laboratory and have reached the stage of commercialization. CE methods for aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, moniliformin and zearalenone have been reviewed by Maragos (1998). The main advantage of CE is the possibility to reach a sensitivity comparable to that of established HPLC methods. Combination of CE with immunoassay makes it possible a simultaneous multi-component analysis due to the high resolving power of CE.

FPI are solution based-assays in which a mycotoxin-fluorophore conjugate (tracer) is used. Applications of FPI assays have been described for detection of deoxynivalenol, fumonisins, aflatoxins, zearalenone and ochratoxin A in cereals, semolina and pasta (Maragos, 2004; Goryacheva et al., 2007). Good correlation have been found between comparative analyses performed by FPI and HPLC. The main advantages of FPI are a high sensitivity and a low matrix interference. The potential speed of FPI assays combined with the portability of commercially available devices, suggests this to be a promising technology for mycotoxin detection. A limit of FPI is that it cannot be used for simultaneous detection of several individual mycotoxins.

SPR is a measure of mass changes that occur in a sensor surface. Applications of SPR assay for detection of DON, fumonisins, aflatoxins, zearalenone and ochratoxin A have been

developed and optimized (Daly et al., 2000; Schnerr et al., 2002; Tudos et al., 2003; van der Gaag et al., 2003). SPR sensitivity for aflatoxin B1 has been demonstrated to be higher than ELISA assay. Studies on naturally contaminated samples showed that SPR results are in agreement with liquid chromatography mass spectrometry (LC-MS) measurements (Tudos et al., 2003; van der Gaag et al., 2003). A technique for the simultaneous detection of four different mycotoxins in a single measurement using SPR commercially available portable equipment was recently reported (van der Gaag et al., 2003).

Emerging challenge of sensors for mycotoxins is represented by the development of non-biologically based binding, such as molecularly imprinted polymers (MIPs) (Maragos, 2004; Logrieco et al., 2005; Krska et al., 2005). Rapid future applications of MIPs are expected if affinity problems are overcome. Mimicking antibodies is the basic idea of MIPs technology. The preliminary results of MIPs technology in zearalenone, deoxynivalenol, and ochratoxin A analysis has been reported (Visconti & De Gerolamo, 2005; Krska & Welzig, 2006). Although the affinity of MIPs are not yet competitive with those of antibodies, this technique offers a good potential for further developments.

Near Infrared (NIR) Spectroscopy, micro system technology tools based on DNA arrays, electronic noses and tongues, biosensors and chemical sensors for the detection of fungal contaminants in feed and food are other emerging, available and promising methods (Larsen et al., 2004; Maragos, 2004; Logrieco et al., 2005; Zeng et al., 2006; Cheli et al., 2008). Infrared (IR) spectroscopy has been continuously evolving, as can be deduced comparing the old mid-IR equipment manufactured in the 1950s and based on dispersive monochromators with the present customized near infrared (NIR) instrumentation. The incorporation of the Fourier transform technique (FT) together with the interferometric spectrometers into the mid-IR instruments has increased the use of this technique in food analysis (Ibañez & Cifuentes, 2001). Although NIR spectroscopy has been used routinely since many years as a rapid method in feed and food industry for determination of constituents such as humidity, proteins, lipids with a precision comparable with that of the official methods of analysis, a limited number of publications concerning mycotoxins and NIR spectroscopy have been reported. This is because the concentration of mycotoxins normally found in feed and food has been considered low for this technique. Recently NIR and mid-infrared (MI) spectroscopy with attenuated total reflection (IR/ATR and FT-IR/ATR) have been used in order to rapidly detect the presence of fungal infection and estimation of fungal metabolites and mycotoxins in naturally and artificially contaminated products (Kos et al., 2002, 2003; Petterson & Aberg, 2003; Berardo et al., 2005; De Girolamo et al., 2009). Multivariate analysis for the extraction of additional information from the recorded spectra gave promising results on the capability of these techniques as tools and models not only for the detection of mould presence, but also for the prediction of the presence of mycotoxins. Chemometric models applied to FT-IR/ATR analysis enabled correct classification of non contaminated and contaminated maize and wheat with deoxynivalenol (Kos et al., 2003; De Girolamo et al., 2009). The developed method enabled the separation of samples with a cut off level for DON of 300 µg/kg, a value below the maximum level and guidance value proposed by the EU for maize and wheat intended for human and animal consumption. Improvements of the classification performance of FT-IR/ATR analysis can be achieved optimising sample preparation procedure and applying particle size analysis to samples (Kos et al., 2007). The use of NIR spectroscopy for the

determination of DON in wheat and fumonisin B1 in maize has been investigated (Petterson & Aberg, 2003; Berardo et al., 2005). It has been shown that it is possible to predict DON concentration in wheat kernels by NIR at levels higher than ca. 400 µg/kg (Petterson & Aberg, 2003), indicating the high potential of IR spectroscopy for accurately predicting the presence or absence of mycotoxins in cereals.

4.1 The analytical approaches miming senses: The example of electronic nose

Further example of rapid methods are those based on electronic senses, which represent an evolution of sensory evaluation traditionally entrusted to the human/animal senses. The evaluation of food and feed in terms of smell, taste, morphology and colour is often overlooked, but contains a lot of information directly related to quality and safety. In particular, the smell and aroma of a food, due to the presence of many volatile chemicals, are sensory parameters of great interest, which can be used as indicators of food quality (Cheli et al., 2007b). Fungal spoilage induces nutritional losses, off-flavours, organoleptic deterioration often associated to mycotoxins formation. Research studies correlated fungal activity with the production of volatile metabolites characterized by gas chromatography mass spectrometry (GC-MS) (Magan & Evans, 2000). These authors conclude that accumulation and pattern of fungal volatiles can be used as indicators of fungal activity and as taxonomic markers in order to differentiate between fungal species and between toxigenic and non toxigenic fungal strains. Since volatile headspace analysis can be evaluated as a whole by the use of electronic nose (EN), this technique is becoming widespread in order to evaluate mould spoilage, quality and safety of food and feed. An EN is an instrument which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odours (Gardner & Bartlett, 1994)(Fig. 1). The array of non-specific chemical detectors interacts with different volatile compounds and provide signals that can be utilised effectively as a fingerprint of the volatile molecules rising from the samples analysed. After the achievement of a fingerprint, the identification and/or quantification of the odours by means of a pattern recognition system become possible.



Fig. 1. An example of electronic nose.

The electronic nose does not distinguish each volatile substance, but express the global odour of a product (Gardner & Bartlett, 1994). This ability, as in the case of other devices as electronic tongue or certain applications of computer image analysis, can enable a general evaluation regarding the “total quality” value of the food and feed analyzed. The process is completed with the aid of appropriate mathematical and statistical methods. As previously cited, the use of EN for evaluating the quality of stored grain has been reported. Sensor technology has been shown to enable to determine the mycological quality of grains. The first type of study carried out with EN technology has been made in order to differentiate between non-infected and infected samples with different species or strain of fungi, through the variation of the metabolic pathway due to the contamination of grains. The ability of EN to differentiate grains and bakery products clean or contaminated (naturally or artificially infected) with different mould species have been demonstrated (Magan & Evans, 2000; Olsson et al., 2000; Balasubramanian et al., 2007; Paolesse et al., 2006). Detection and differentiation between mycotoxigenic and non-mycotoxigenic strains of *Fusarium* spp. using volatile production profiles evaluated by EN has been also reported (Keshri & Magan, 2000; Magan & Evans, 2000; Falasconi et al., 2005; Presicce et al., 2006; Sahgal et al., 2007). Further developments of studies carried out with EN technology have been made in order to evaluate the possibility of using fungal volatile metabolites as indicators of mycotoxin presence (Campagnoli et al., 2009b). Results from a study carried out on naturally contaminated barley samples showed that it was possible to use volatile compounds to predict whether the OTA level in samples was below or above 5 µg/kg; seven of 37 samples were misclassified (Olsson et al., 2002). EN analysis enabled correct classification of naturally contaminated maize with aflatoxins (Campagnoli et al., 2009a, 2009b; Cheli et al., 2009b). EN analysis was applied to wheat in the case of naturally DON contaminated samples (Tognon et al., 2005; Dell’Orto et al., 2007; Campagnoli et al., 2009b). A simple analytical protocol, combined with the application of the CART (Classification and Regression Tree) model and PCA (Principal Component Analysis) for the selection of variables and the classification of samples was used in another paper (Campagnoli et al., 2011). Results obtained indicated that the EN equipped with ten MOS (Metal Oxide Semiconductor) sensors array allows the classification of naturally contaminated samples on the basis of DON content into three classes on the basis of the European Union limits for DON in unprocessed durum wheat: (a) non-contaminated; (b) contaminated below the limit (DON < 1,750 µg/kg); (c) contaminated above the limit (DON > 1,750 µg/kg); with a validated prediction error rate of 0% when a 20-sample dataset was considered. (Campagnoli et al., 2011). The same model was used with a 122-sample dataset, 9 contaminated and 113 non-contaminated samples, more faithfully reproducing a real-life situation characterised by unbalanced classes. Although, classifying performance was lower than in the 20-sample dataset case, reasonable results were achieved, with a validated prediction error rate of 3.28% (Table 3). Four errors were computed in prediction; however, none of the contaminated samples were misclassified as non-contaminated, avoiding the worst eventuality under in-field conditions.

Less information is available regarding quantification capability of electronic nose in order to predict mycotoxins concentration in cereals. Tests were conducted on DON levels in barley and wheat. Positive correlation was found between electronic nose data and reference concentration of DON (Olsson et al., 2002). However the performance of the regression model on prediction was quite low (PRESS =0.65, R² =0.63, adjR² =0.63) (Tognon et al., 2005; Dell’Orto et al., 2007).

	Total true samples		Misclassification matrix (Samples fitted assignment)			Validated misclassification matrix (Samples predicted assignment)		
			Assigned class			Assigned class		
			a	b	c	a	b	c
Class a	113	<i>rate</i>	112 0.991	0 0.000	1 0.009	111 0.982	1 0.009	1 0.009
Class b	6	<i>rate</i>	0 0.000	6 1.000	0 0.000	0 0.000	5 0.833	1 0.167
Class c	3	<i>rate</i>	0 0.000	0 0.000	3 1.000	0 0.000	1 0.333	2 0.667

Table 3. EN use for DON analysis in wheat: performances of classification for a 122-samples dataset. Class a) samples non-contaminated; Class b) samples below the legal limit; Class c) samples above the legal limit (modified from Campagnoli et al., 2011).

5. Conclusion

The plan of an effective sampling procedure for food and feed contaminants' detection or quantification represents a complex challenge for operators. Special attention has to be paid when matrices are coarse and contaminants are characterized by a non uniform distribution, as in the case of mycotoxins in cereal commodities, that represent the most important worldwide human and animal food and feed resources. Under these conditions, sampling uncertainty dominates in the final uncertainty result, then the choice of expensive, precise, sensible, specific analytical method could result an inefficient strategy. Instead, the adoption of a rapid, low cost but high sample throughput analytical approach able to test a high number of samples can represent a better option. This is one of the most important reason for which R&D regarding these analytical approaches and statistical data analysis specifically dedicated merits further implementation. Fearn (2009) states that "*The safest policy is to use the simplest method you can, and within that the simplest model you can, avoiding the temptation to add a lot of extra complexity for a small gain in performance*". Therefore, some analytical methods reveal further useful characteristics for screening purposes. For example, methods miming senses, i.e electronic nose, that, by means of rapid and simple analytical protocols, can provide a general description regarding the quality of complex matrices of interest. Then, samples could be classified and a limited selected number submitted to more expensive and time-consuming quantitative analyses with useful costs reduction.

6. References

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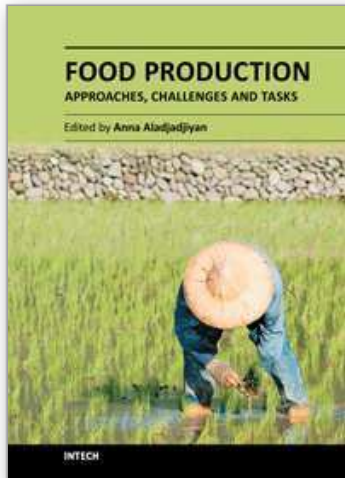
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