

# Infrared spectroscopy applied to identification and detection of microorganisms and their metabolites on cereals (corn, wheat, and barley)

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## 1. Introduction

Over the last several years, mycotoxins, which are the metabolites secreted by fungi, have been the subject of numerous studies. These eukaryotes play a major ecological role in the life cycle of plants. For some fungi, the role of saprophyte places them at the heart of ecosystem dynamics [Alexopoulos et al., 1996].

Some 350 mold species produce a large range of secondary metabolites (over 300, of which approximately 30 are toxic) [Fremy et al., 2009] and represent a potential danger for animal and human health in addition to causing significant losses for the cereals industry [Le Bars et al., 1996]. The effects of mold are well illustrated by decreases in crop and livestock yields, public health problems, or write offs on the international cereal market [Le Bars, et al., 1996]. The United Nations Food and Agriculture Organization estimates annual global losses from mycotoxins at 1 billion tons of foodstuffs [Fao, 2001]. The primary organisms impacted by mycotoxins are plants and about 25% of agricultural crops worldwide are currently contaminated by these metabolites [Charmley et al., 2006].

In response to these significant economic and health risks, global nontariff barriers (i.e., specific food-safety standards imposed on imported products) were erected to control commercial trade based on the mycotoxic quality of foodstuffs. These measures generate significant economic and material losses for countries that export contaminated foodstuffs, either because their cargo is refused or because of a reduction in prices. To limit these consequences, farmers and the food industry strive to reduce the presence of mycotoxins in their products. Therefore, producers and processors are searching for alternative analytical methods to determine, in a quick, simple, and inexpensive manner, the risk of their products containing fungi or mycotoxins. The use of infrared spectroscopy—a mature technology—to monitor foodstuffs could respond to this need.

In this chapter, we focus on mycotoxins found mainly in wheat, barely, and corn and that have been studied in the international literature; namely, deoxynivalenol, fomonisins, and aflaltoxin B1.

## 2. Advantages of using infrared spectroscopy to manage fungal and mycotoxic risk for wheat, barely, and corn

Fungus can be detected by microbiological methods involving visual, microscopy, and microbial-cultural methods.

Conventional methods of mold detection are based on direct observation by eye or by microscope of thalli, contaminated foodstuffs, or microbial cultures. These methods are time consuming and require viable samples and a good deal of expertise. Counting methods are difficult to apply to fungi because,

during their reproduction, a spore generates a mycelium that can in turn divide itself into tens of individuals. Furthermore, a fungal contamination may not be visible at the surface of grains [Hirano, et al., 1998, Pearson, et al., 2001].

Other methods are based on molecular biology or on the detection of antigens specific to given molds. Organisms, either dead or alive, can be detected by the polymerase chain reaction (PCR) by copying a large number of DNA sequences that are originally present in small quantities (with a multiplicative factor on the order of  $10^9$ ). By amplifying certain genes of toxigenic strains, PCR serves as a tool to determine the potential risk of the presence of mycotoxins. Various researchers have tested PCR to detect *Fusarium* contamination in corn [Jurado et al., 2006; Jurado et al., 2005; Nicolaisen et al., 2009]. These methods are rapid, sensitive, and can be automated. They are good qualitative methods (e.g., good selectivity) but offer only average precision in quantitative terms (they are called "semiquantitative"). These techniques are thus very reliable, provided the fungal strain to be detected is known beforehand, and so are used as referential methods. With such methods, a grain is deemed of suitable microbiological quality if less than 10 000 germs of the storage flora per gram of grain are detected.

New approaches are based on detecting constituents and fungal metabolites. Such approaches exploit the fact that molds have specific characteristics that distinguish them from other eukaryotes. These characteristics include the regulation of certain enzymes, the synthesis of lysine amino acid by a particular metabolic route, extremely structural characteristics (e.g., the Golgi apparatus), and genetic characteristics (e.g., haploid). From among these attributes, two types of compounds can be used as indicators of a fungal contamination.

The secreted compounds are synthesized compounds such as soluble carbohydrates (e.g., disaccharide trehalose and polyhydric alcohols such as mannitol or arabitol) or products of the metabolization of complex carbons such as volatile aldehydes, alcohols, ketones, spores, primary metabolites, secondary metabolites (i.e., volatile compounds). The last item gives rise to the characteristic fungal odor and is often detected by an electronic nose. For nonvolatile compounds, other tools such as infrared spectroscopy seem better suited.

The structural compounds of mold can also be used for their detection. The main polysaccharides of the cell wall of mold are the  $\alpha$  et  $\beta$  (1-3) glucans, as well as chitin. Ergosterol is a component of fungal cell membranes.

Chitin may absorb infrared light, making it useful for infrared spectroscopy. [Nilsson, et al., 1994; Roberts et al., 1991]. The main inconvenience in using this component as an indicator of fungal contamination is that chitin is not limited to fungi; it is found in insects, diatoms, arachnids, nematodes, crustaceans, and several other living organisms [Muzzarelli, 1977]. In addition, it may take different forms, each of which requires a specific detection technique. Roberts et al. [Roberts, et al., 1991] estimates the quantity of mold on barely by detecting this molecule but also detects glucans by near-infrared spectroscopy.

Ergosterol, however, is more specific to mold. This molecule, which may still be called provitamin D<sub>2</sub>, is a C<sub>24</sub>-methylated sterol (and is part of the subgroup of organic compounds that are soluble in lipids) and is found in the cell membranes of yeasts and filamentous fungi. This molecule is not found in animal cells [Verscheure et al., 2002] and is in the minority among the sterols found in higher plants [Pitt et al., 1997] and insects [Weete, 1980]. Griffiths et al. [Griffiths et al., 2003] demonstrated that ergosterol is the primary sterol found in molds: it represents 95% of the total sterols, with the remaining 5% being ergosterol precursors from *Leptosphaeria maculans*. This specificity makes this molecule a potential tracer

of fungal activity. It is generally agreed that the ergosterol content of grains must be less than a given threshold; the limit for corn is 8 µg/g.

### 3. Infrared spectroscopy to detect fungal and mycotoxic contamination of wheat, barely, and corn

#### 3.1. Background and methods

The first application of infrared spectroscopy to detect microorganisms dates from the 1950s [Miguel Gomez et al., 2003]. In these applications, the spectrometers were calibrated depending on the method of dosing the fungi or mycotoxins. In the 1980s, Fraenkel *et al.* [Fraenkel et al., 1980] and Davis *et al.* [Davies et al., 1987] published their first works on the detection by infrared spectroscopy of fungal contamination (*Botrytis cinerea* and *Alternaria tenuissima*), but the application of this tool to detecting mold really grew in the 1990s. This growth was due to the fact the existing agronomic models required collecting a significant amount of information in the field, making this approach unsuitable for routine use. In addition, industry required nondestructive techniques to assess the health safety of crops. Therefore, several research teams used infrared spectroscopy to detect mold and mycotoxins on cereals, which could be done concomitantly with the quantification of other parameters such as protein content, humidity, etc.

One method proposed to determine the fungal or mycotoxin content is to quantify the total fungal biomass. Toward this end, ergosterol is used as a fungus marker [Castro et al., 2002; Saxena et al., 2001; Seitz et al., 1977; Seitz et al., 1979]. Very often, this type of study is coupled with a study of the mycotoxin content and fungal units (colony-forming units or CFU). Indeed, the quantity of fungi is not proportional to the quantity of mycotoxins; it is possible to have small quantities of fungi but large quantities of mycotoxins, and vice versa. Indeed, fungi may disappear after secreting its toxins, either because of the evolution of the mycoflora or because of the application of chemical treatments. In addition, certain strains are more toxic than others. Two conclusions exist from the work on this subject: Some researchers find a correlation between the mycotoxin content, the ergosterol content, and/or the fungal units [Lamper et al., 2000; Le Bouquin et al., 2007; Miedaner et al., 2000; Seitz et al., 1977; Wanyoike et al., 2002; Zill et al., 1988] whereas the others find no correlation or cannot make categoric conclusions [Beyer et al., 2007; Diener et al., 1982; Gilbert et al., 2002; Nowicki 2007; Penteado Moretzsohn De Castro et al., 2002; Perkowski,et al., 1995].

Covering the last 20 years, we count over 20 articles dealing with the use of infrared spectroscopy (primarily near-infrared) to detect molds and mycotoxins in wheat, barely, and corn. Because some of the work in infrared spectroscopy deals both with the detection and the identification of mycotoxins, we separate the articles into three groups. Table 1 is for molds, Table 2 compiles the trials dealing with deoxynivalenol (DON), fumonisins (FUMs), and B1 aflatoxins (AF1). Finally, Table 3 contains articles in which the authors worked simultaneously on the fungal and mycotoxic aspects. Each table lists the matrix studied (wheat, barely, corn), the apparatus, the content ranges, the performance of the models, the principle conclusions, and the characteristic wavelengths.

#### 3.2. Principle conclusions

##### 3.2.1 Identification and quantification of fungi

For identification of fungi, the performances, given in terms of percentage of correct classification, are very satisfactory because they exceed 77%. Each study identifies the peaks or spectral zones related to the growth of fungi or to the damage inflicted on the grains by fungi.

Moreover, the performance of the quantification of ergosterol always gives enticing results.

### 3.2.2. Quantification of mycotoxins deoxynivalenol, fumonisins, and aflatoxins

Mycotoxins are present in quantities too small (on the order of parts per million) for direct detection. Their detection is thus associated with a complex ensemble of information related to the growth of the fungus on the cereal; notably with modifications of the protein or carbohydrate level (starch, cellulose, etc.).

Regarding the capabilities of infrared spectroscopy to quantify mycotoxins, the conclusions differ from one author to another. In general, when dealing with deoxynivalenol, the performance is higher than when dealing with fumonisins. Yet despite this, even if the quantification of mycotoxins appears possible, it is not sufficiently precise to be used in the field. Indeed, the standard error of prediction (SEP) is too large with respect to the regulatory limits—notably European limits. Moreover, to work under conditions of realistic of toxin levels, the main avenues for improvement of these studies may be the following:

- Increasing the number of samples;
- Increasing the annual variability (samples often come from a single harvest);
- Allowing a natural contamination of the grains (artificial contamination does not account for all the natural parameters of contamination, notably so for multicontaminations);
- Accepting a range of mycotoxin levels more adapted to the reality in the field (the ranges are often very narrow);
- Acquiring spectra not grain-by-grain, but from entire lots of grain. Indeed, it is more difficult to assign a global mycotoxin level to a lot, because the distribution of mycotoxins is very heterogeneous, as is the case for the molds that synthesize the mycotoxins;
- Using a set of test samples (the proposed performances are often cross validated and are thus better than they would be for an external test);
- Displaying the ratio of standard error of prediction to sample standard deviation (RPD).

Thus, instead of quantification, several studies propose a classification of cereals samples as a function of the mycotoxin level. This qualitative approach works better and, at least until the quantitative models are improved, seems the most conclusive for applications in realistic conditions. Note also that, even if the SECs, SECVs, and SEPs are improved for the quantifications, these models are developed based on chemical benchmarks that themselves have non-negligible standard errors.

## 4. Conclusion

Infrared spectroscopy offers multiple advantages. This tool requires no preparation and does not use toxic products. It enables nondestructive analysis of the samples. Moreover, it is a rapid, low-cost technique that can be used online. The sample cell is resistant and inexpensive (glass or quartz). Finally, infrared spectroscopy enables multiparametric analyses and a large range of robust devices are available.

Models to classify cereals samples as a function of the type of fungus present or its level, or the presence of mycotoxins, represent an attractive tool to determine the fungal and mycotoxic risk in the food industry; for example, in the field or in the silo. Regarding the quantification of mycotoxins, chemometrics, which is a field in constant progression, may one day deliver performances that meet agricultural and industrial needs.