ALLERGEN AND MYCOTOXIN DETECTION

Comparing and contrasting the various testing methods

by Dr. M. Hikmet Boyacioglu

Codex Alimentarius' General Standard for the Labeling of Prepackaged Foods states, "The following foods and ingredients are known to cause hypersensitivity and shall always be declared: cereals containing gluten; i.e., wheat, rye, barley, oats, spelt or their hybridized strains and products of these; crustacea and products of these; eggs and egg products; fish and fish products; peanuts, soybeans and products of these; milk and milk products (lactose included); tree nuts and nut products; and sulphite in concentrations of 10 mg/kg or more."

"The Food Safety Modernization Act is transforming the nation's food safety system regarding allergen controls and responsibilities," said Richard E. Goodman, PhD, research professor, and Jamie Kabourek, dietitian and resource manager, University of Nebraska Food Allergy Research and Resource Program. "In the U.S., the major allergens include milk, eggs, fish, crustacean shellfish (shrimp, crab, lobster), peanut, soybean, tree nuts (almond, walnut, cashew, etc.) and wheat. Recent evidence suggests that sesame seed may be added to the list. Verification of ingredients from international sources can be challenging. In developing or modifying products, companies must consider allergenic sources, including purified or concentrated proteins. New gluten-free products pose potential risks for those with celiac disease."

The Food Allergy Research and Resource Program (FARRP) maintains both an allergen and a celiac protein database to evaluate proteins from genetically engineered sources and novel food ingredients that are risks of food allergy or celiac disease (www.allergenonline.org). According to Goodman and Kabourek, food companies should develop an Allergen Control Plan (ACP) and employee training focusing on acquisition, storage, handling, processing, packaging and identification of allergenic foods and ingredients.

"In March 2016, a major flour miller was subjected to a class II recall when it was discovered that their wheat was contaminated with peanuts," said Tim Hendra, director of corporate accounts, Neogen Corp. "The source was determined to be the rail cars, which had previously hauled peanuts."

He added that this one example underscores a major

Moldy corn kernels are a sign that mycotoxin may be present. Photo by Adobestock.

challenge bakery and snack food manufacturers face, specifically commodity commingling. Many flour ingredients are grown on the same farms as allergenic ingredients; they may be harvested at the same time, with shared equipment, and stored in the same bins. Unfortunately, it is nearly impossible to separate allergenic from non-allergenic foods at the agricultural level. Peanuts in wheat flour, soy in wheat flour, soy in corn, and wheat in oats are just a few examples of food allergen cross-contamination risks.

Arthur Tatham, professor in Food Science and Nutrition, Cardiff Metropolitan University, U.K., said that as patterns of cereal consumption change, so do the patterns of allergy and intolerance.

"For example, increasing wheat consumption in Eastern Asia has led to a rise in wheat-related intolerances and allergies in wheat-consuming populations," he said.

Detection and quantification methods are necessary not only to comply with food labeling requirements, but also to prevent costly product recalls, improve consumer protection, ensure consumer confidence, and brand name protection.

Several technical approaches for the detection of allergens in cereals are available. The methods employed either target the allergen itself (protein or glycoprotein) or a marker (specific protein or DNA fragment) that indicates the presence of the offending food. Detection of the allergen is not always feasible since this often may be present in trace amounts, may vary in abundance between cultivars, or may be masked by the food matrix. Moreover, the sensitivity of the method utilized may influence detection.

EnviroLogix offers several solutions for detecting mycotoxins. Photo courtesy of EnviroLogix.

Polymerase chain reaction (PCR) methods is the dominant DNA-based technique used for the detection of allergenic ingredients and the only DNA-based method for which commercial test kits are available. PCR is a relatively fast and inexpensive method for identifying DNA. It is an alternative method to ELISA that is routinely used for the identification and quantification (real-time PCR) of genetically modified organisms, pathogens and food-related plant and animal species. PCR also has been used in breeding programs for the detection and negative selection of wheat cultivars with poor bread-making quality, demonstrating that it can be an efficient alternative to standard procedures of separation for early screening of useful wheat genotypes with good bread-making quality.

The Enzyme-Linked Immunosorbent Assay (ELISA) method uses antibody reagents developed to either specific allergens or protein fractions from allergenic foods. The ELISA technique is the most common method used in food analysis to detect and quantify allergens due to its high precision, simple handling, and good potential for standardization. The most frequent method for the analysis of gluten in food products for the determination of gluten in the milligram/kilogram range is based on ELISA. It is generally an accurate test and is considered highly sensitive and specific (accurate) and compares favorably with other methods used for the detection of substances in the body. One of the greatest advantages of the ELISA test is the ability to obtain quick and accurate results. According to Romer Labs, ELISA is the most widely applied method for the detection and quantification of food allergens. However, although many samples can be analyzed at the same time, these samples can only be tested for one analyte. Furthermore, in highly processed foods, they may give false-negative results or reduced quantifications.

A third technique has been applied to analysis of allergens in foods using quantitative mass spectrometric (MS) methods. According to Romer Labs, mass spectrometry is considered the allergen testing method having the most potential for future improvements due to its outstanding reliability, sensitivity, and the potential to perform multi-allergen analysis. However, it noted that mass spectrometry needs highly skilled personnel, and the initial investment costs are high due to the expensive instrumentation. Furthermore, the time to result will always be much longer than for immuno methods.

Lateral flow devices or strip tests are other techniques to determine allergens in foods. They are inexpensive, easy to use, do not require laboratory equipment, and give results usually in a few minutes. However, most strip tests are only qualitative and rely on antibodies as recognition elements. Therefore, they suffer from the same

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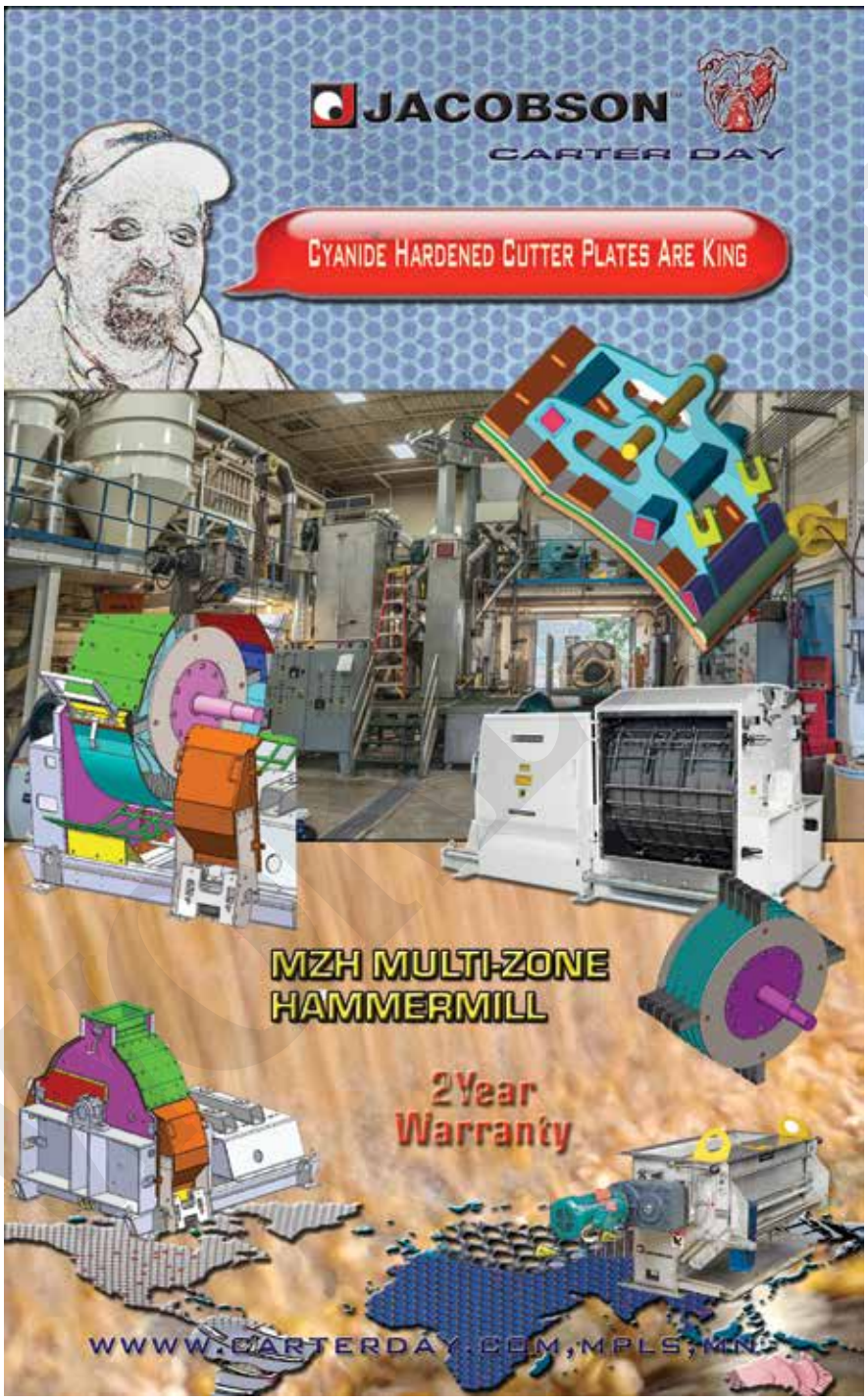
Carlos A. Campabadal, PhD, IGP Institute, Kansas State University



problems as ELISA tests with highly processed food.

Electrophoresis also has been used separately or in combination with other methods to study cereal allergens.

Arjon J. van Hengel, European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Italy, said the scientific community is challenged to develop analytical methods that can detect all the allergenic foods that are listed in the legislation. This illustrates that many methods need to be developed to detect all allergenic ingredients that require a mandatory declaration reliably. Methods that can detect multiple allergens in a single analysis are needed to tackle this problem. Furthermore, the reliability of the methods needs to be investigated. Validation studies and prescience testing are required. Guidance on the organization of validation studies as well as proficiency testing rounds is forthcoming, but currently, only methods for a limited number of allergenic foods have been used in validation studies and proficiency test rounds.

MYCOTOXIN TESTING

Cereals and cereal-based products are essential food staples in many countries, as well as being used in animal feed and many industrial applications. However, their contamination with mycotoxins is a major concern due to adverse effects of mycotoxins on human health. Many countries set legal standards for mycotoxins in food and feed. The determination of whether foodstuff complies with the legal standards for mycotoxins set by relevant regulatory agency requires a sample of the foodstuff to be taken and analyzed.

Mark MacBeath, senior product man-

ager in PerkinElmer's food segment, noted that while it depends on the growing season, geographical region and recent weather, generally the most common mycotoxins have been aflatoxins in corn, soy and wheat, and DON (vomitoxin) in wheat and corn.

"Mycotoxins are secondary metabolites produced by certain molds present in grains and other agricultural products that are toxic to animals and humans," said Carlos A. Campabadal, PhD, IGP Institute, Kansas State University. "It is very common in the animal production industry to find health issues related to mycotoxins. Therefore, the feed industry must be very careful on how it manages and handles its grain when producing animal feed."

Campabadal said currently there are only two mycotoxins that are known to be produced during grain storage. These two mycotoxins are aflatoxins and ochratoxins that are produced by molds from the *Aspergillus* family. The rest of the known mycotoxins are produced in the field during the growing phase of grain.

"Feed millers should consider implementing different strategies to effectively control mycotoxins on their grain," he advised. "As a first step, during grain or feed ingredient purchasing, maximum allowable limits of mycotoxins should be emphasized on the purchasing contracts to reduce any potential amounts. Second, when receiving grain or its co-products, proper testing should be performed to quantify any potential levels, and if they exceed the allowable limits, different considerations should be implemented like denying entry of the load or segregating it to avoid any cross-contamination or to make any other logistical decision."

Breck O. Parker, PhD, chief scientific officer of EnviroLogix, added, "Mycotoxin and allergen test data contribute to a growing spectrum of invaluable agricultural meta data used to drive better business decisions and ultimately meet increasing consumer demand for transparency."

Ryan Whipkey, product manager, life sciences, EnviroLogix, emphasized the importance of feed ingredients quality on high-quality feed production and noted that "rapid tests exist to measure nutritional content and natural toxins such as mycotoxins at the point of delivery. For mycotoxins, co-occurrence detection in crop commodities and processing co-products is on the rise, and mycotoxin test kit providers have responded by making kits available that can test the same sample extract, al-

Grain facilities can detect and quantify five major mycotoxins using a single, solvent-free sample extraction through VICAM's recently launched Myco 5-in-1 lateral flow strip test solution. Photo courtesy of Vicam.

lowing feed mills to process trucks at a faster rate. These quantitative mycotoxin tests are inserted into an instrument that processes test results and makes them available for digital distribution."

"A growing challenge for grain operations is the need to screen and quantify more than one mycotoxin in a single inbound or outbound shipment," said Patricia Jackson, market development manager, VICAM. "Global regulatory limits and end-user quality specifications (such as corn millers, biofuels, feed or pet food manufacturers) often require grains to meet strict maximum contamination levels for aflatoxin, fumonisin, vomitoxin (DON), zearalenone and ochratoxin prior to acceptance."

MYCOTOXIN TEST METHODS

Mycotoxin distribution in grains is largely non-homogeneous since often only a reduced amount of materials in bulk is contaminated at very high levels. Therefore, in the evaluation of the level of mycotoxin in bulk, the error associated with the sampling step is the most relevant contribution to the overall uncertainty. Accordingly, to obtain a laboratory result that reflects the level of toxin present in a lot of product, standard sampling and laboratory preparation procedures should be followed.

BIOMIN classifies mycotoxin analysis methods as reference testing methods: Thin Layer Chromatography (TLC); Gas Chromatography (GC); High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography-Mass Spectrometry (LC/MS); and rapid testing methods (Lateral Flow Test; Enzyme-Linked Immunosorbent Assay, ELISA and Fluorometry).

- **TLC:** The TLC technique represents the most economical method in mycotoxin analysis and is widely used in developing countries. However, its sensitivity is limited, it does not work for all mycotoxins and it uses solvents that regard ecological hazard. New detection techniques for TLC have been developed as alternatives to traditional TLC scanners.
- **GC:** Gas chromatography-based methods represent the most widely employed techniques for the determination of trichothecenes, as deoxynivalenol and nivalenol, since these compounds do not intensely absorb in the UV–visible range and are nonfluorescent so that the use of mass spectrometry (GC/MS) is in some cases crucial in the identification step.
- **HPLC:** A wide variety of methods for most mycotoxins are based on HPLC. The advantages of HPLC methods include excellent performance characteristics, low detection level, and safety for the operator. However, it is time-consuming, expensive and requires a trained technician.
- **LC-MS:** The LC-MS technique is relatively popular, mainly due to well-established, sensitive and reliable LC techniques with fluorescence detectors with the ability to measure the natural fluorescence of toxins. PerkinElmer's QSight Triple Quad 400 Series (a LC/MS/MS system) provides advanced confirmation testing and analyzes multiple types of mycotoxins — like aflatoxin, vomitoxin, zearalenone, fumonisin, T-2 and Ochratoxin A — in grain products such as wheat, corn, soy and more at large processors and contract labs, said MacBeath. Amin Mousavi Khaneghah et al., 2019, reviewed published studies on mycotoxins in cereal-based products for 24 years (1983–2017) and reported that the lowest and highest number of published reports was associated with DON and total aflatoxin in some of the cereal-based products, respectively. They also found that the liquid chromatography-electrospray ionization-tandem mass spectrometry

(LC-ESI/MS) was categorized as the most implemented technique for mycotoxin detection.

- **Lateral flow strip tests:** Grain facilities can detect and quantify five major mycotoxins using a single, solvent-free sample extraction through VICAM's recently launched Myco 5-in-1 lateral flow strip test solution, said Jackson. She added, "This streamlined approach reduces consumables use and test time up to 80% compared with traditional methods, providing a truly simple way for global grain operations to verify quality and safety." Looking at numerous mycotoxins at the same time is becoming necessary to monitor risks. Neogen's new Raptor detection platform is like *"three readers in one."* The unique design allows testing for three toxins at the same time with an automated time and temperature control system and bar code read of test strips. Neogen's Q+ mycotoxin platform with Raptor will be adding Ergot test this fall, said Pat Frasco, director of corporate accounts, Neogen. MacBeath said PerkinElmer's new AuroFlow AQ Afla strip test with QuickSTAR Horizon strip reader helps lab professionals, technicians and farmers conduct first-round screening for aflatoxins in corn (including B1, B2, G1, and G2) down to 2-300 ppb detection levels, in six minutes. He also said this solution features a single-step, water-based extraction method with lateral flow testing at room temperature — enabling safe and easy sampling without incubators and centrifuges. The handheld reader is battery operated and rugged, supporting flexible in-field testing. Results are easy to view on the reader's menu-driven color touchscreen and then stored and archived for further reference and audit trails.

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Patricia Jackson, market development manager, VICAM

- **ELISA:** The enzyme-linked immunoassay (ELISA) is probably the most commonly used antibody-based assay for mycotoxin detection. However, the disadvantage of this technique is represented by the possibility of obtaining false positives when cross-reaction happens as well as false negatives when very low levels of contamination are to be checked. Therefore, the ELISA methods are suggested as low-cost screening methods prior to confirmatory analysis, to lower the costs considerably when a high number of samples must be analyzed.

FUTURE DEVELOPMENTS IN MYCOTOXIN TESTING

Modern techniques rely on liquid and gas chromatography coupled with mass spectrometry, making this one of the most popular and secure analytical methods that can detect different mycotoxins simultaneously. HPLC linked with fluorescence detection (HPLC-FLD) as one of the non-MS chromatographic techniques has been adopted by the Association of Official Analytical Chemists (AOAC) and European Standardization Committee (CEN) International for the identification and quantitation of mycotoxins.

Regarding future developments in mycotoxin testing, MacBeath said: "We see faster and better methods coming to the fore that will increase the accuracy of screening while reducing training times for part-time technicians. In addition, there may be some automated imaging and texture methods for screening purposes on the horizon that will deliver much faster results, enabling companies to clear trucks through the facility faster."

The reliability of the measurements is crucial in food and agricultural areas, particularly in the case of undesirable toxic compounds such as mycotoxins. Quality control principles for mycotoxin analysis are common to other trace analyses. Follow-up of good laboratory practices is vital for reliable measurements. WG

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