

MYCOTOXIN ANALYSIS IN TREE NUTS

Regulatory, Technology, and Economic Considerations

For tree nut processors and traders, the results of contaminant testing are a key determinant of market share and profitability. Tree nuts that meet the most stringent purity standards command higher prices and fulfill the toughest trade requirements while products that fall short are sold at lower margins and may lose access to the most lucrative markets. Given the far-reaching economic impact of contaminant levels, the price of test results that misrepresent them could prove unaffordable.

These costly miscalculations are most likely to occur when the contaminant in question is subject to exceptionally tight controls, and the technical challenges of accurately quantifying it are particularly complex. Few contaminants fit this description better than aflatoxins, a toxic threat that looms large on regulatory radar screens in more than 100 countries.

AFATOXIN CONTAMINATION: ENVIRONMENTAL RISK FACTORS, CHEMICAL PROPERTIES, AND HEALTH IMPLICATIONS

Aflatoxins are the most well known and extensively studied of mycotoxins, a class of toxic mold metabolites that pervade the natural environment. Other mycotoxins such as ochratoxin A (OTA) can occur in concert with aflatoxins, significantly increasing the overall toxicity of the contamination. The molds that produce aflatoxins and other mycotoxins can infect nuts while they're still on the tree as well as during post-harvest processing, transportation, and storage.

The environmental factors that promote aflatoxin contamination are often difficult and sometimes impossible to control. The hot, humid conditions that favor the spread of aflatoxin-producing molds in pre-harvest crops are a case in point. Whether high outdoor temperatures and moisture levels are an endemic climate feature or a normal variation in the weather, these natural phenomena lie beyond the capacity for human intervention. Although climate control systems can help prevent these conditions from occurring indoors during storage and transport, seemingly minor lapses in maintenance like a malfunctioning fan, leaks, or condensation can turn a warehouse or vehicle interior into a virtual mold incubator. Forces of nature can also lead to widespread contamination by heightening the vulnerability of nuts to fungal invasion. Drought stress, violent storms, and insect infestations expose nuts to this risk by damaging their health and physical integrity. While strategies like irrigation and pest management can mitigate some of this damage, these measures may prove inadequate when conditions are unusually severe. According to a number of climate change models, such conditions could eventually become the new global reality, as temperatures, pest reproduction rates, and the frequency of droughts and other extreme weather events continue to increase.¹

While post-harvest practices like drying and sorting can stop the spread of toxigenic molds, the mycotoxins they produce are by nature much harder to eliminate. These highly stable chemical compounds can persist in crops after mold removal and are remarkably resistant to traditional detoxification strategies, such as heat processing and irradiation. In experiments by the Food and Agricultural Organization (FAO) of the United Nations, irradiation destroyed only 30 percent of the aflatoxin content in a contaminated sample, and oil and dry roasting left as much as 30 to 50 percent intact.²

Efforts to minimize aflatoxin levels in susceptible commodities have intensified as the world has grown increasingly aware of the threat that this group of mycotoxins poses to human health. A naturally occurring mixture of four types of aflatoxin—B₁, B₂, G₁, and G₂—is of particular concern to food safety officials. Chronic exposure to low doses of this toxic mixture can lead to an increased risk of liver cancer, reduced immune function; and liver, kidney, and neurological damage. The most commonly found of the four types, aflatoxin B₁, is one of the most potent human carcinogens ever identified. For this reason, many countries mandate B₁ limits that are lower than those that are typically set for total aflatoxins (i.e., the sum of the individual levels of B₁, B₂, G₁, and G₂).

THE ECONOMIC CHALLENGES OF THE GLOBAL REGULATORY SCENE

While regulations that restrict aflatoxins to parts-per-billion levels are the general rule, maximum levels and the complexity of standards vary from country to country. Aflatoxin standards in two of the most potentially profitable tree nut markets—the European Union and parts of Asia—are both tougher and more individualized than the 20 ppb action level for all tree nuts set by the U.S. FDA. (See Table 1.)

Table 1: Maximum levels for aflatoxin B₁ and total aflatoxins in ready-to-eat tree nuts in the European Union, South Korea, and Japan*

Region	Aflatoxin B ₁ (ppb)	Total aflatoxins (B ₁ +B ₂ +G ₁ +G ₂) (ppb)
EU	Almonds/pistachios: 8 Hazelnuts/Brazil nuts: 5 All other nuts: 2	Almonds/pistachios, hazelnuts, Brazil nuts: 10 All other nuts: 4
US	All nuts: 20	All nuts: 20
Japan		All nuts: 10
South Korea	All nuts: 10	All nuts: 15

*Comprehensive data on aflatoxin and other mycotoxin limits in these and other commodities and countries around the world are available from VICAM's Global Mycotoxin Regulations Tool. <www.commodityregs.com>

These strict control measures continue to pose a challenge for exporting countries. Reports from the EU's Rapid Alert System for Food and Feed show that in the three-month period between June and August of 2015, aflatoxin levels in more than 50 incoming tree nut shipments exceeded legal limits.² The repercussions of such violations are prohibitively expensive. According to almond industry estimates, the cost of reprocessing, replacement shipments, warehousing, and other expenses can amount to as much as \$10,000 for a single rejected consignment and can climb much higher if that consignment is destroyed.³

As significant as these monetary losses are, they pale beside the long-term economic damage that compliance failures can inflict on individual brands and regional industries. From a loss of consumer confidence to restrictive import conditions or an outright trade ban, the consequences of exceeding international aflatoxin limits can severely compromise an industry player's ability to compete in the global marketplace.

For the top tree nut producing nations (see Table 2), the financial implications of this issue are huge. The leading exporter of this commodity, the United States earned \$2.7 billion from tree nut exports in 2014, with the bulk of those exports going to the EU and emerging Asian markets.⁴

Table 2: Major growing regions for 8 varieties of tree nuts

Tree nut variety	Major growing regions
Almonds	California, Spain
Pistachios	Iran, California
Pecans	U.S. (Georgia, Southwest)
Walnuts	China, California
Hazelnuts	Turkey, Italy
Cashews	Vietnam, India, Nigeria, Ivory Coast
Brazil nuts	Bolivia, Venezuela
Macadamia nuts	Australia, South Africa, Hawaii

REDUCING UNCERTAINTY: THE VALUE OF HIGH-PERFORMANCE TESTING TECHNOLOGY

With so much riding on such low maximum limits, the ability to accurately and reliably determine aflatoxins at ppb levels has never been more important. For tree nut businesses and the laboratories that test their products, that ability requires a sampling plan and a test method that satisfy the rigorous criteria of standards organizations and regulatory agencies. These testing approaches are the most widely trusted because they're

specifically designed to address the most frequent causes of variable and inaccurate test results.

Sampling plans that meet official requirements are generally based on the principle of representative sampling. This principal calls for pooling many small incremental samples taken from different locations throughout a product lot to form an aggregate sample, which is then finely ground in a food mill to obtain a homogeneous sample for testing. The tree nut sampling plan prescribed by European officials calls for a 20 kg aggregate sample for all nuts, one 20 kg analytic sample for nuts destined for further processing and two 10 kg analytic samples for ready-to-eat nuts. The number and size of incremental samples that make up the aggregate sample vary according to the lot's weight with a minimum of ten 20 kg samples for loads that weigh less than 1 ton.⁵ The purpose of these procedures and requirements is to reduce the chances of either overestimating or underestimating the overall concentration of aflatoxins in a lot. The high risk of such errors arises from the tendency of mycotoxins to cluster in pockets, or "hotspots," in widely scattered location in a large shipment.

To further minimize the risk miscalculating mycotoxin levels, AOAC International, a major player in promoting global agreement on official measurement standards and test methods, confirmed the use of high-quality immunoaffinity (IA) columns for sample cleanup. A leading choice for compliance testing in Europe as well as in the United States, AflaTest® IA columns from Massachusetts-based test developer VICAM offer the performance advantages of an AOAC- and USDA-approved test kit.

AflaTest not only optimizes samples for analysis, but also streamlines the complex and laborious procedures traditionally used for sample extraction and purification. Tailored to the matrix characteristics of almonds, cashews, walnuts, pistachios, and pecans, the cleanup process for tree nuts consists of a few easy steps. As a diluted and filtered sample is passed over the column, the highly specific monoclonal antibodies in the column strongly bind with the aflatoxins in the sample. After washing the column with water to flush out matrix impurities, methanol is applied to detach the aflatoxins from the antibodies. To complete the process, the methanol containing the aflatoxins is then collected and mixed with water. By maximizing the recovery of aflatoxins while removing matrix components that possess similar properties, this cleanup method significantly decreases the risk of false negatives and false positives. The simplicity of the process also helps minimize the occurrence of procedural errors.

For high-stakes regulatory applications, VICAM recommends coupling AflaTest column cleanup with liquid chromatography (LC). Widely used in reference laboratories, the combination of AflaTest and highly sensitive and exacting instrumental techniques such as HPLC and UPLC® helps ensure that processors' and traders' aflatoxin assessments are consistent with those of government agencies. By accurately, reliably, and precisely measuring individual levels of aflatoxin B₁, B₂, G₁, and G₂ as well as total aflatoxins, this advanced analytic method makes it possible to confidently determine compliance with regulations everywhere around the globe. The method is efficient, too, with an average time of less than 30 minutes for HPLC analysis.

AflaTest can also be used with a fluorometer to prescreen samples in laboratories, as well as to check aflatoxin levels during shipment, handling, and storage. Fluorometric testing requires no special training and determines aflatoxin concentrations at ppb levels in less than 15 minutes. With higher limits of detection than instrumental analysis and limited to measuring total aflatoxins, this method offers a highly affordable approach to boosting laboratory efficiency; facilitating rapid, informed buying and selling decisions; and monitoring contamination to prevent its spread. For even faster and easier measurement of ppb concentrations of total aflatoxins, VICAM offers Afla-V®, a quantitative strip test that takes as little time as 5 minutes to develop and enables users to view a clear digital readout of the results on the screen of a portable device.

SUMMARY

Vigilant aflatoxin surveillance has become standard operating procedure for a growing number of tree nut enterprises. The industry continues to invest heavily in aflatoxin monitoring programs to ensure access to key markets and sustain the trust of today's increasingly safety-conscious consumers. Frequent testing is not only cost-effective business insurance, but also an ethical, socially responsible corporate policy that helps protect the health of customers and the livelihoods of industry stakeholders around the globe. Whether it's measured in dollars or public goods, the industry's return on that investment depends on the quality and soundness of its aflatoxin testing approach.

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