Prevention of aflatoxin in pistachios

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Awareness that undesirable levels of aflatoxin in food and feed may have serious consequences for human and animal health is increasing. Aflatoxins, probably the most studied and widely known mycotoxins, were first noted in the early 1960s. They are among the most potent mutagenic substances known; there is extensive experimental and epidemiological evidence that they induce liver cancer (WHO, 1998). The term aflatoxin refers to a class of chemical compounds of related structure; among these aflatoxin B1 is considered to be the most potent carcinogen.

The major aflatoxin-producing fungi are Aspergillus flavus and Aspergillus parasiticus. Under favourable temperature and humidity conditions these fungi grow on certain foodstuffs, most commonly groundnuts, dried fruit, tree nuts (almonds, pecans, walnuts, pistachios and Brazil nuts), spices and a range of cereals (especially maize). Production of aflatoxin is optimal at relatively high temperatures, so contamination is most acute and widespread in warm, humid climates. Although contamination is generally considered to be a problem in tropical and subtropical regions of Africa, Asia and Latin America, aflatoxins have also been found in temperate countries of Europe and North America (FAO, 1979, 1982).

CONTAMINATION

Fungal contamination and subsequent production of aflatoxin can occur in crops in the field, at harvest, during postharvest operations and in storage. The rate and degree of contamination are dependent on temperature, humidity and soil and storage conditions. Prevention, particularly by excluding or reducing toxigenic mould growth and toxin production in susceptible food crops, is the most effective way to restrict mycotoxin contamination. In practice, this can be accomplished by reducing fungal infections in growing crops through rapid drying and correct storage of the harvested crops, the use of effective anti-mould preservatives and adherence to proper postharvest processing, transport and distribution practices.

Many mycotoxins, including aflatoxins, can form during the growing stages of certain crops. Climate, sources of fungal inoculum (or suitability of the fungal substrate), potential insect vectors and the plant response (or the plant susceptibility to fungal infestation) can interact to result in specific mycotoxin occurrence. Damage caused by insects can expose susceptible tissues to colonization by toxigenic fungi with subsequent mycotoxin formation.

Following harvest and during shipment and storage of agricultural commodities, toxigenic mould growth and potential mycotoxin production are influenced by many factors including moisture level, temperature, aeration, infestation by insects and other microorganisms, storage time, chemical treatments, spore infection density and storage conditions (especially leakage of water or condensation).

By far the most critical environmental factors determining whether a substrate will support mould growth are moisture content, temperature and time. Thus, drying, proper storage and suitable transportation are of prime importance in prevention.

The prevention of mycotoxin contamination in storage is largely a matter of strict moisture control of the crop. There must be no insect activity, as insects can create favourable microclimates for toxigenic fungal growth; no moisture migration; no condensation or water leaks; and no rodent activity, as the moisture level could be increased by urination. In summary, conditions which restrict fungal growth will almost invariably limit or exclude mycotoxin production.

Where harvesting occurs in dry weather, mycotoxin contamination does not usually reach alarming proportions. It becomes a problem where harvesting is done in very humid weather. In many developing countries, the combination of insufficient drying equipment coupled with humid atmospheric conditions results in unacceptable levels of aflatoxin in harvested groundnuts, tree nuts and other foods.

AFLATOXIN IN PISTACHIOS

What conditions favour mould growth and aflatoxin production in pistachio nuts and how can this be prevented? Many Aspergillus species infect nuts and cause decay of the kernels before harvest. For example, it was reported that up to 13 species were isolated from pistachio kernels from

1 Adapted from the author’s presentation at the Pistachio '97 Conference, 27-28 October 1997, Rome, Italy.
orchards in Iran and 14 species from orchards in the United States (California) (Doster and Michailides, 1994a).

The shells of most pistachio nuts split naturally in the orchard prior to harvest. Fortunately, the hull covering the shell usually remains intact, protecting the kernel from invasion by moulds and insects (see Figure). Nuts that are poorly protected by hulls are most prone to contamination in the orchard. Sometimes the hull is attached to the shell so that it splits with the shell, exposing the kernel to moulds and insects. This is called an “early split”. In some countries the proportion of early splits can be as high as 30 percent. In a study done in the United States, approximately 1 to 5 percent of the nuts were early splits. When early splits were examined in one study about 20 percent of the samples (50 nuts each) were found to be contaminated with aflatoxin, while there was no contamination in nuts with hulls that remained intact in the orchard (Doster and Michailides, 1995; Sommer, Buchanan and Fortlage, 1986). Although the importance of early splitting for mould, aflatoxin and insect contamination is well established, very little is known concerning when early splits occur and become contaminated. This knowledge could aid in timing the application of either chemical or biological control treatments.

Besides early splitting, hull rupture may occur from very late harvesting, bird damage and cracking. The navel orange worm (Amyelois transitella) commonly infests nuts with ruptured hulls and such infestation has been associated with very high levels of aflatoxin. Nuts infested with navel orange worm pose less of a danger because they are easier to eliminate by sorting, and consumers are likely to reject them.

Early split nuts that are not infected in the orchard may become infected during transport and handling. High humidity and high temperature within bulk bins provide ideal conditions for the infection of early split nuts, which dramatically increases the incidence and level of aflatoxin contamination, until nuts are mycologically stabilized by drying or refrigeration.

The development of Aspergillus mould in pistachio litter has been related to the increase of Aspergillus inoculum in the orchard and results in greater numbers of mouldy and mycotoxin-contaminated pistachio nuts. Certain practices such as burying or removing litter may decrease colonization by Aspergillus moulds in the orchard. Another possible treatment for pistachio litter includes the application of microorganisms that limit the development of Aspergillus mould. Further research is needed to determine if any of these practices would reduce Aspergillus colonization of litter enough to decrease the numbers of mouldy and mycotoxin-contaminated nuts harvested (Doster and Michailides, 1994b).

**RECOMMENDATIONS OF THE CODEX ALIMENTARIUS COMMISSION**

The Codex Recommended International Code of Hygienic Practice for Tree Nuts (CAC/RCP 6-1972) (FAO/WHO, 1994b) provides basic hygienic requirements for orchard, farm processing (shelling and hulling) and commercial shelling and in-shell operations. It covers environmental sanitation in growing and food production areas, sanitary harvesting, transportation, plant facilities and operating requirements, operating practices and production requirements, in particular raw material handling, inspection and sorting, preparation, processing and storage. Prevention and control measures should allow compliance with the Codex standard for unshelled pistachio nuts (CODEX STAN 131-1981) (FAO/WHO, 1994a) as well as with respective provisions for food additives and contaminants laid down in Codex texts.

**MAXIMUM LEVELS**

The need for setting maximum levels of aflatoxins in foods and feeds is generally recognized. Several countries, particularly some industrialized ones, have already set specific regulations. Limits for aflatoxin B₁ in foodstuffs range from 0 to 30 µg/kg, while those for total aflatoxins range from 0 to 50 µg/kg (FAO, 1997). In 1994-1995, FAO, in collaboration with the Dutch National Institute of Public
GUIDELINES FOR PREVENTING MOULD CONTAMINATION AND AFLATOxin PRODUCTION

A prevention programme should consider various steps from cultivation through harvesting, postharvest handling, drying, postdrying storage, processing, postprocessing storage, transportation and marketing (Danesh et al., 1979; Mojtahedi et al., 1978; Thomson and Mehyd, 1978; Crane, 1978).

Harvesting

- Tree nuts, including pistachios, should be harvested as soon as possible after maturation to avoid quality loss and to minimize problems involving fungal attack and infestation with insects, especially the navel orange worm.
- Signs of optimum harvesting time are: separation or ease of separation of the hull from the shell; decrease in fruit removal force; drying of hull and kernel.
- Pistachios should not be shaken to the ground to avoid contamination of open shells by litter and soil.
- In hand picking of young trees, clean tarps should be used to avoid contact of the nuts with soil and litter.

Postharvest handling

- Pistachios should be hulled and dried soon after harvest to minimize shell staining and decay and to ensure safety. If temporary storage of fresh pistachios at the dehydration plant is necessary, they should be cooled and held before hulling at 0°C and relative humidity lower than 70 percent.
- Pistachios should be sorted before cold storage to remove defective nuts, leaves, twigs and other foreign materials.
- Fumigation with methyl bromide or phosphine can be used to control insects in stored nuts. It may be necessary to repeat fumigation periodically depending on the conditions and duration of storage.

Drying

- In the two-stage process, the hulled nuts are first dried for about three hours in a column dryer or a rotating drum dryer (air temperature around 82°C) to reach kernel moisture of 12 to 13 percent. Nuts are then transferred to a flat-bottomed grain bin (second stage) where they continue drying with warm air (temperature less than 49°C) for one to two days to reach a moisture content of 5 to 6 percent.
- In the single-stage process, a self-unloading bin dryer is used at air temperature of 60° to 66°C. Drying time is eight hours.

Postdrying storage

- Dried nuts are usually stored in bins, silos or other bulk storage containers for a few weeks or several months before final processing and preparation for market.
- Optimum storage conditions of 10°C or lower and 65 to 70 percent relative humidity must be maintained to minimize deterioration, including the growth of Aspergillus flavus.
- Protection against insects is also essential during the storage period.

Processing

- Nuts are sorted, using visual or light reflectance electronic sorting techniques, to eliminate those with defects (nuts with adhering hulls, stained and moldy nuts).
- Empty or partially empty pistachios are separated by an air stream, and unsplit pistachios by floatation in water.
- Shells are cracked for extraction of kernels when desired.
- Nuts are sorted to eliminate those with possible aflatoxin contamination.
- Nuts are salted, flavoured and roasted.
- Nuts are packaged: in-shell nuts, shelled nuts and nut meat (broken kernels) in various types and sizes.

Postprocessing storage

- Water activity should be kept below 0.70 at 25°C (moisture content less than 7 percent).
- Relative humidity should be kept below 70 percent to avoid any significant mould growth.
- Temperature should be kept between 0° and 10°C depending on expected storage duration. The lower the temperature, the longer the storage life.
- Low oxygen (less than 0.5 percent) helps maintain flavour quality and effects insect control. Exclusion of oxygen is usually done by vacuum packaging or by packaging in nitrogen.

Health and Environment, carried out a global survey of mycotoxin regulations which covered some 90 countries (FAO, 1997). The survey indicated that only France, Greece and Jordan have set specific regulations for aflatoxin levels in pistachio nuts (Table 1). In several countries, the maximum tolerable level for aflatoxin in pistachios is covered under the category “nut products” (Table 2). In 36 other countries, pistachio nuts are covered under the category “all foods”.

The setting of internationally agreed maximum tolerable
Involving the loss of the food altogether or without severely compromising the availability of major food supplies.

In 1996 and 1997, JECFA re-evaluated aflatoxins. At its June 1997 meeting, the committee concluded from a quantitative risk assessment on aflatoxins that there is no significant difference in risk to human health between the maximum levels of 10 µg/kg and 20 µg/kg for aflatoxin B1 in food (WHO, 1998). The Codex Committee on Food Additives and Contaminants has responsibility for proposing guideline levels for naturally occurring toxicants in foods and feeds. It will examine the JECFA recommendation, and its proposals will then be reviewed by the Codex Alimentarius Commission for possible adoption as international standards.

From a regulatory standpoint, aflatoxins are considered unavoidable contaminants in foods since they cannot be prevented or eliminated by current good agricultural practices. National regulations should not jeopardize the availability of food crops at reasonable prices, and the implications for farming, especially subsistence farming in developing countries, must also be considered. Thus exposure of the population to some level of aflatoxins is tolerated.

The total value of pistachio nuts traded in the international market exceeds US$500 million (Moaven, 1998). Harmonization of regulations at the global level helps importing countries to secure regular supplies of commodities and helps exporting countries to find markets for their products. A balance must be sought between the needs of importing and exporting countries, without jeopardizing the consumer’s health. The setting of unnecessarily low guideline levels for aflatoxin may result in a technical barrier to trade.

**CURRENT NEEDS**

Aflatoxin contamination of pistachio nuts is undoubtedly a serious problem for many producing countries. The European Union’s 1997 ban on pistachio imports from Iran because of high levels of aflatoxin in some shipments exemplifies the seriousness of this problem. Unless concrete measures are taken by the pistachio-producing countries and by traders to address the problem, the situation may worsen. Prevention of mould growth and of aflatoxin production throughout the pistachio production and distribution chain is the key solution. The systematic and organized application of integrated phytosanitary management (IPSM) in orchards to eliminate, if not reduce, mould infection during the cultivation stage, coupled with a Hazard Analysis and Critical Control Point (HACCP) approach to prevent contamination during the harvest and postharvest stages, should lead to the desired results (Kader, 1992). This will not be possible without proper training of all actors in the

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**Table 1**

<table>
<thead>
<tr>
<th>Country</th>
<th>Aflatoxin Level (µg/kg)</th>
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<tbody>
<tr>
<td>France</td>
<td>B1 1</td>
</tr>
<tr>
<td>Greece</td>
<td>B1 5; B1 + G1 + G2 10</td>
</tr>
<tr>
<td>Jordan</td>
<td>B1 15; B1 + G1 + G2 30</td>
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**Table 2**

<table>
<thead>
<tr>
<th>Country</th>
<th>Aflatoxin Level (µg/kg)</th>
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<tbody>
<tr>
<td>Australia</td>
<td>B1 + B2 + G1 + G2 15</td>
</tr>
<tr>
<td>Canada</td>
<td>B1 + B2 + G1 + G2 15</td>
</tr>
<tr>
<td>Cyprus</td>
<td>B1 5; B1 + B2 + G1 + G2 10</td>
</tr>
<tr>
<td>(to be confirmed)</td>
<td></td>
</tr>
<tr>
<td>Israel</td>
<td>B1 5; B1 + B2 + G1 + G2 15</td>
</tr>
<tr>
<td>New Zealand</td>
<td>B1 + B2 + G1 + G2 15</td>
</tr>
<tr>
<td>Philippines</td>
<td>B1 + B2 + G1 + G2 20</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>B1 + B2 + G1 + G2 4</td>
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According to FAO estimates, up to 25 percent of the world’s food production is lost at postharvest level. Such losses include those caused by mould and mycotoxin contamination. FAO is greatly concerned with the impact of mycotoxin contamination of foods because of its negative effects on human health, food trade and food availability and consumption. For years, FAO’s Food Quality and Standards Service has provided the Organization’s member countries with advice on policies and strategies for preventing and controlling mycotoxin contamination; its activities include efforts to harmonize mycotoxin tolerance levels in foods and feeds and respective methods of sampling and analysis.

The implementation of prevention programmes may include not only the prevention of mycotoxin formation in agricultural products (through better farm management practices, use of fungus- and toxin-resistant varieties, proper irrigation and fertilizer practices, pest control, crop rotation, improved postharvest techniques – including drying and storage practices – and adequate use of chemicals), but also their removal through detoxification or decontamination. Also required are routine surveillance; regulatory measures to control the flow of mycotoxin-contaminated commodities in national and international trade; and information, education and communication activities. Each of these approaches forms a part of an overall strategy to minimize the impact of mycotoxin contamination.

In dealing with mycotoxin problems, FAO has organized, in collaboration with WHO and the United Nations Environment Programme (UNEP), international meetings where experts exchange their views, identify priorities at the regional or international level and propose strategies for further action. Two joint FAO/WHO/UNEP international conferences on mycotoxins have been held, in 1977 and 1987.

Some recommendations from the last conference referred specifically to prevention measures, such as:

- the development of feasible and practical methods to prevent preharvest contamination of food crops and to improve postharvest practices at both local and large-warehouse levels, so as to prevent fungal infection and insect damage;
- the coordination of research activities on issues pertaining to mycotoxin toxicity, incidence, prevention and elimination;
- the development of feasible, economical and safe means of decontamination of mycotoxin-contaminated food commodities and products.

FAO has also organized regional meetings in order to review and adapt the proposed international strategy. Workshops were held in Asia (1990), in Africa (1991) and in Latin America (1991) to assist in identifying specific regional needs and priorities. The Asian workshop, for instance, referred to the need for a bold Asian initiative for the 1990s to observe the “decade of prevention and control” with activities designed to minimize mycotoxin contamination in food.

At the national level, FAO has assisted a number of developing countries in strengthening their capabilities to prevent and control the contamination of foods by mycotoxins. These activities have been carried out through field projects addressing specific problems related to mycotoxin prevention, surveillance and control. Of the 200 projects in FAO’s Special Action Programme for the Prevention of Food Losses, some 50 have included components for mycotoxin control. For example, the Southeast Asia project, involving 13 countries, introduced appropriate postharvest technologies related to mycotoxin prevention, with emphasis on drying facilities.

FAO promotes the dissemination of technical information on mycotoxin-related issues. Several publications and training aids on various aspects of mycotoxin prevention programmes, including methods of sampling and analysis, have been prepared and distributed widely. A training syllabus was developed in 1990 for use in short-term courses on aflatoxin analysis in Africa. Publications created as part of project activities include a directory of mycotoxin prevention and control institutions in Asia. A global compilation of maximum tolerable levels of mycotoxins in foods and feeds has been recently issued (FAO, 1997).

Further research is needed to explore other means of preventing mould infection and aflatoxin production. These could include prevention of contamination in the field through the use of biological control agents that are pathogenic to Aspergillus flavus, such as yeast or harmless bacteria, and that could act as ecologically friendly bio-competitors (FAO, 1979; Katz, 1996). Scientists are exploring a genetically engineered antibody to toxin production which could be applied when conditions are favourable to toxin production. In other words, the mould would grow but would not produce toxin. Finally, research is needed to produce pistachio varieties that are more resistant to aflatoxin.

On the regulatory side, efforts should be made to establish, as soon as possible, internationally agreed guideline levels for aflatoxin in pistachio nuts which would be accepted by all parties so as to eliminate possible technical barriers to trade. Such guideline levels should be based on sound scientific evidence and on realistic risk assessment. The recent JECFA work on this subject is a breakthrough and provides hope for a positive development in the near future.

**REFERENCES**


Aflatoxin contamination in food and feed supplies may have serious consequences for human and animal health. Aflatoxins are commonly associated with groundnuts, dried fruit, tree nuts, spices and cereals. Contamination is most acute and widespread in the warm, humid areas of Africa, Asia and Latin America. However, aflatoxins are found in temperate areas of North America and Europe as well. Contamination can occur in crops in the field, at harvest, during postharvest operations and in storage. The rate and degree of aflatoxin contamination are dependent on temperature, humidity and soil and storage conditions.

Early splitting, hull rupture and damage from birds and insects make pistachios susceptible to contamination. Research is needed to prevent mould infection and aflatoxin production and to produce pistachio varieties that are more resistant to aflatoxin.

Prevention of mould growth and of aflatoxin production is crucial and requires proper training of farmers, processors, warehouse keepers, traders and transporters. Food control officials and extension workers should be closely involved in these efforts. The Codex Alimentarius Recommended International Code of Hygienic Practice for Tree Nuts provides basic hygienic requirements for orchard, farm processing (shelling and hulling) and commercial shelling and in-shell operations. Prevention and control measures should allow compliance with the Codex standard for unshelled pistachio nuts (Codex Standard 131-1981).

From a regulatory standpoint, aflatoxins are considered unavoidable contaminants in foods since they cannot be prevented or eliminated by current good agricultural practices. Thus exposure of the population to some level of mycotoxins is tolerated. The need for setting maximum levels of aflatoxins in foods and feeds is generally recognized. Several countries, particularly some industrialized ones, have already set specific limits, ranging from 0 to 30 µg/kg for aflatoxin B₁ in foodstuffs and from 0 to 50 µg/kg for total aflatoxins.

The setting of internationally agreed maximum tolerable levels for aflatoxins in food and feed is of global importance. Recently, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that there is no significant difference in risk to human health between the maximum levels of 10 µg/kg and 20 µg/kg for aflatoxin B₁ in food. Unnecessarily low guideline levels for aflatoxin may result in a technical barrier to trade.
La contaminación de alimentos y piensos con aflatoxinas puede tener graves consecuencias para la salud de los seres humanos y de los animales. Las aflatoxinas suelen estar asociadas con el maní, las frutas secas, las nueces, las especias y los cereales. Aunque la contaminación es más acusada y está más extendida en las zonas cálidas y húmedas de África, Asia y América Latina, también se encuentran aflatoxinas en zonas templadas de América del Norte y Europa. La contaminación puede ocurrir cuando los cultivos están aún por recoger, durante la cosecha, después de ésta y en el curso del almacenamiento. La velocidad y el grado de la contaminación con aflatoxinas dependen de la temperatura, la humedad, el tipo de suelo y las condiciones de almacenamiento.

La rajadura temprana de los pistachos, la ruptura de la cáscara y los daños causados por aves e insectos hacen que las nueces queden más expuestas a la contaminación. Es necesaria una investigación para prevenir la infección con mohos y la producción de aflatoxinas y para obtener variedades de pistachos que sean más resistentes a éstas.

La prevención de la proliferación de mohos y la producción de aflatoxinas es muy importante y exige una capacitación apropiada de agricultores, elaboradores, almacenistas, comerciantes y transportistas. Los funcionarios encargados del control de los alimentos y los agentes de extensión deben participar también activamente en esas actividades. El Código Internacional Recomendado de Prácticas de Higiene del Codex Alimentarius para las nueces producidas por árboles tiene por objeto proporcionar los requisitos higiénicos básicos para los huertos, para las operaciones que se realizan en las granjas (descortezado y pelado) y/o las operaciones comerciales, tanto si se trata de quitar la cáscara como si se refieren a las nueces rodeadas de su cáscara. Las medidas de prevención y control harán posible el cumplimiento de la norma del Codex para los pistachos con cáscara (Norma 131-1981).

Desde el punto de vista reglamentario, las aflatoxinas se consideran contaminantes inevitables de los alimentos, dado que no pueden prevenirse ni eliminarse con las buenas prácticas agrícolas actuales. Por consiguiente, se tolera la exposición de la población a cierto nivel de aflatoxinas. Por lo general se reconoce la necesidad de establecer niveles máximos de aflatoxinas en los alimentos y los piensos. Varios países, entre ellos algunos industrializados, han establecido ya reglamentaciones específicas. Los límites para la aflatoxina B₁ en los alimentos varía de 0 a 30 µg/kg, con un margen de variación de 0 a 50 µg/kg para las aflatoxinas totales.

La fijación de niveles máximos admisibles aceptados internacionalmente para las aflatoxinas en los alimentos y los piensos tiene una importancia mundial. El Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios llegó recientemente a la conclusión de que un nivel máximo de 10µg/kg o 20 µg/kg de aflatoxina B₁ en los alimentos no daba como resultado una diferencia significativa en el riesgo para la salud humana. Unos niveles de referencia innecesariamente bajos para las aflatoxinas pueden crear obstáculos técnicos al comercio. ♦