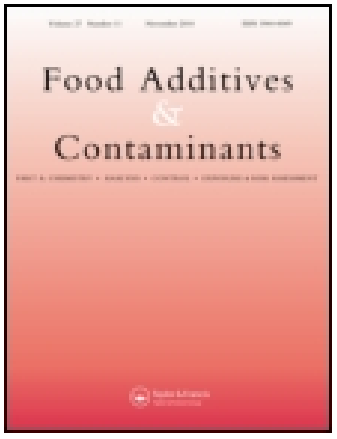

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Mycotoxins in botanicals and dried fruits: A review

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Mycotoxins in botanicals and dried fruits: A review

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Abstract
Botanicals are used in many countries for medicinal and general health-promoting purposes. Numerous natural occurrences of mycotoxins in botanicals and dried fruits have been reported. Aflatoxins or ochratoxin A (OTA) have been found in botanicals such as ginseng, ginger, liquorice, turmeric, and kava-kava in the USA, Spain, Argentina, India, and some other countries, while fumonisins have been found in medicinal wild plants in South Africa and in herbal tea and medicinal plants in Turkey. Zearalenone was identified in ginseng root. Dried fruits can be contaminated with aflatoxins, OTA, kojic acid, and, occasionally, with patulin or zearalenone. One main area of concern is aflatoxins in dried figs; bright greenish yellow fluorescence under ultraviolet light is associated with aflatoxin contamination. OTA in dried vine fruits (raisins, sultanas, and currants) is another concern. There are also reports of aflatoxins in raisins and OTA in dried figs, apricots, dried plums (prunes), dates, and quince. Maximum permitted levels in the European Union include $4\,\mu g\,kg^{-1}$ for total aflatoxins in dried fruit intended for direct consumption and $10\,\mu g\,kg^{-1}$ for OTA in dried vine fruit. This review discusses the occurrence of mycotoxins in botanicals and dried fruits and analytical issues such as sampling, sample preparation, and methods for analysis. Fungal contamination of these products, the influence of sorting, storage, and processing, and prevention are also considered.

Keywords: *Botanicals, dried fruits, garlic, ginseng, ginger, capsicum, liquorice, raisins, figs, fumonisins, ochratoxin A, aflatoxins*

Introduction

Botanical products and various dried fruits are in great demand in the health food markets. As people of differing nationalities live in the same communities, ethnic foods become increasingly popular and available. Varying processing and storage conditions can provide mould growth and mycotoxin development. This review will discuss the incidence and occurrence, methods of analysis, and prevention of occurrence of mycotoxins in botanicals and dried fruits. There have been many investigations of the occurrence of mycotoxins in these products. Dried fruits, particularly dried vine fruit (raisins, sultanas and currants), and figs (Drusch and Ragab 2003; Drusch and Aumann 2005) have been of considerable interest.

Mycotoxins in botanicals

The term 'botanicals' simply means plants or plant products. Usually botanicals have been understood as medicinal plants and botanical supplements. In many cases it is difficult to distinguish between these two categories. Numerous botanical products enter markets around the world as foods and as dietary supplements. The food or herbal supplements may be whole plants, plant parts or plant extracts. Medicinal plants have been used as medicines for centuries and some of them are now used in developed countries as alternative or complementary medicines. Traditional remedies have been used in China for over 5000 years, and currently it is estimated that half of all healthcare delivered in China is based on traditional Chinese

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medicines (Shaw 1998). A 1998 report shows that 89% of people in developing countries rely on traditional herbal medicines. These are widely available to consumers worldwide and some of them are used as alternative medicines. Herbal and other 'alternative' treatments are also popular in the developed world, being used by about 50% of Australians and 33% of Americans (Shaw 1998). In industrialized countries it is believed that between 30 and 50% of the population regularly use herbal medicines and/or vitamin and mineral supplements. The most common reasons given for taking supplements are 'it makes me feel better' and 'to live longer'. In the USA since 1994, with the passage of the Dietary Supplement Health and Education Act (DSHEA) (US Food and Drug Administration (FDA) 1994), herbal supplements easily enter commercial markets. Herbal supplement usage has increased tremendously since the passage of the DSHEA. The DSHEA states that the government cannot prohibit the sale of dietary supplements unless the products prove to be unsafe. Consequently, they are sold over the counter in drug stores, supermarkets, health supply shops and through the internet. Some are used daily by consumers for various reasons. In many cases, the functions and toxicities of the inherent bioactive compounds in the botanicals are largely unknown. Contamination with chemicals such as mycotoxins, heavy metals, pesticides and synthetic drugs, microorganisms such as bacteria and fungi, or undeclared constituents can contribute to adverse human health problems (Schilter et al. 2003).

Incidence and occurrence

In spite of the long history and wide use of botanicals as foods and traditional medicines, there are few publications on their contamination with moulds and mycotoxins compared with numerous publications on the contamination of grains and oil seeds. Several surveys of toxigenic moulds in botanicals have found high levels of *Aspergillus*, *Penicillium* and *Fusarium* spp. (Abeywickrama and Bean 1991; Halt 1998; Rizzo et al. 2004). While the presence of moulds might not be correlated with the presence of mycotoxins, there are reports of aflatoxins, ochratoxin A (OTA) and fumonisins in medicinal plants, tea and other botanicals.

Raw materials for medicinal use and herbal supplements are frequently contaminated with toxigenic fungi generated from the soil, or the plants themselves, during harvesting or in storage. Contamination with mycotoxins produced by these fungi could pose human health problems. There are more than 20 000 botanicals available in the market, but very few studies on occurrence of mycotoxins in botanicals. It may be due to the lack of methods of analysis suitable for determining mycotoxins in botanicals as the use of botanicals as supplements is relatively new. This section covers mycotoxin contamination in medicinal plants and some of the common botanical supplements such as garlic, ginseng, ginger, capsicum, turmeric, liquorice roots, kava-kava (also known as kava) and red mould rice (RMR) (also known as red yeast rice).

Medicinal plants

Almost all countries have indigenous plant species that have been used to prevent illness or cure diseases. Surveys of fumonisins in medicinal plants from 2001 to 2006 indicated that widespread low levels of fumonisin B_1 (FB_1) occurred in Portugal, Turkey, and South Africa. In Portugal, a total of 87 samples (69 samples of four different medicinal plants and 18 samples of black tea) were purchased from supermarkets and analysed by liquid chromatography (LC) for FB_1 and fumonisin B_2 (FB_2) (Martins et al. 2001). Fifty-five samples were contaminated with FB_1, but no FB_2 was found in any of these samples. Sixteen samples of the black tea and 39 samples of the medicinal plants contained FB_1 at levels ranging from 80 to 280 $\mu g\,kg^{-1}$ and from 20 to 700 $\mu g\,kg^{-1}$ FB_1, respectively. In Turkey, a total of 115 herbal tea and medicinal plant samples were analysed using an LC method. FB_1 was detected in two samples at 160 and 1487 $\mu g\,kg^{-1}$ and no FB_2 was found (Omurtag and Yazicioğlu 2004). In South Africa, 30 medicinal plants were analysed for FB_1 and aflatoxin B_1 (AFB_1) using immunoaffinity column (IAC) clean-up and LC with fluorescence (FL) detection (Sewram et al. 2006). None of the plant extracts contained AFB_1. Four samples were positive for FB_1 at levels ranging from 8 to 1553 $\mu g\,kg^{-1}$ and its presence was confirmed by LC-tandem mass spectrometry (LC-MS/MS).

Studies from China, Egypt, India, Sri Lanka, Thailand, Malaysia, and Indonesia reported finding AFB_1 in medicinal plants and one study from India found citrinin (CIT) in addition to AFB_1. Three of 19 traditional Chinese medicines contained aflatoxins (up to 28 $\mu g\,kg^{-1}$; Yang et al. 2005). In Egypt, nine of the 31 herbs and medicinal plants analysed contained average AFB_1 levels of 49 $\mu g\,kg^{-1}$ by LC with ultraviolet light (UV) detection (Selim et al. 1996). In India, 14 out of 15 different drug plant samples collected from storehouses contained AFB_1 at 0.1–1.2 $\mu g\,kg^{-1}$ (Roy et al. 1988). In another study in India, 36 out of 60 samples of seeds of medicinal plants were positive for AFB_1 at levels ranging from 20 to

1180 μg kg^{-1} and 11 were positive for CIT at 10–760 μg kg^{-1} (Roy and Kumari 1991). OTA was found at levels up to 2340 μg kg^{-1} in 55 out of 129 herbal samples destined for the preparation of Ayurvedic medicines (Roy and Kumar 1993). Other work from the same group in India found 23 out of 50 samples of liver curative herbal medicines contained AFB_1; the maximum level was 2230 μg kg^{-1} in *Asparagus racemosus* (Kumar and Roy 1993). In Sri Lanka, 500 μg kg^{-1} AFB_1 was detected by thin-layer chromatography (TLC) in one of six Asian medicinal plants, *Aerra lanata* (Abeywickrama and Bean 1991). In Thailand, five out of 28 herbal medicinal products were contaminated with aflatoxins at 1.7–14.3 μg kg^{-1} using an IAC/LC method (Tassaneeyakul et al. 2004). In Malaysia and Indonesia, 16 of the 23 traditional herbal medicines jamu and makjun analysed by an IAC/LC method contained a low level of AFB_1 (0.3 μg kg^{-1}) (Ali et al. 2005).

In Croatia, seven samples of medicinal plant material were analysed for AFB_1, OTA, and zearalenone (ZON). Only a trace amount of OTA was found in one sample (Halt 1998). There was no mention of the method of analysis and the limit of detection (LOD).

In Italy, 27 aromatic herbs, 48 herbal infusions and medicinal plants were analysed for aflatoxins by LC with post-column derivatization and FL detection; none of the samples was contaminated, even if it originated from tropical countries (Romagnoli et al. 2007). In Japan, 49 powdered herbal drugs were analysed for aflatoxins, sterigmatocystin and OTA; mycotoxins were not detected in any samples (Hitokoto et al. 1978). Aflatoxins were found in one out of ten tablets of *Cascara sagrada* dried bark sold in Argentina as well as in two out of nine samples of the raw material (Rizzo et al. 1999).

Garlic

Garlic is the most widely used botanical supplement in the USA. People take garlic supplements for the following uses: antibacterial function, antifungal function, to lower blood pressure, to lower cholesterol levels, to improve the circulation, as a cardioprotective, as an anti-oxidant, and for coughs and colds, stomach conditions and cancer treatment. Many of these uses have not been evaluated by sound scientific studies. There are only two reports on analysis for aflatoxin in garlic. In the UK, a survey of mycotoxins in ethnic food including four garlic samples was conducted. No aflatoxins were found at >0.1 μg kg^{-1} (Patel et al. 1996). Another study found one garlic powder to contain 3.3 μg kg^{-1} total aflatoxins (MacDonald and Castle 1996). The same study showed that aflatoxin levels in spiced sauces were not reduced by cooking or microwave heating.

Ginseng

Ginseng has been used in Chinese medicine for more than 5000 years. The term 'ginseng' means 'man root' in Chinese. The botanical name *Panax* means 'heal all' in Greek. There are only two reports of finding aflatoxins in ginseng: one in ginseng roots and one in ginseng supplements. In the USA, 11 simulated wild and 12 cultivated ginseng root samples were analysed by D'Ovidio et al. (2006). All cultivated roots were found to be aflatoxin-free. Two of the simulated wild roots contained aflatoxins at 15.1 and 15.2 μg kg^{-1}. One mouldy ginseng root purchased from a grocery store was contaminated with aflatoxins at 16 μg kg^{-1}. Ten ginseng products were purchased from herbal supply stores, grocery stores and drug stores in the USA (Trucksess et al. 2007). Three products were found to contain about 0.1 μg kg^{-1} AFB_1 and four products had OTA at levels ranging from 0.4 to 1.8 μg kg^{-1}. The remaining samples contained no aflatoxins or OTA (<0.1 μg kg^{-1}).

ZON has been found in ginseng. Crude extracts of dried ginseng roots from four sources were screened for ZON content using direct competitive enzyme-linked immunosorbent assay (ELISA) (Gray et al. 2004). ZON in one wild-crafted *Panax quinquefolius* was 680 μg kg^{-1} and in two *P. ginseng* and one *P. quinquefolius* were 183, 386, and 177 μg kg^{-1}, respectively. However, ZON levels in the same four extracts measured by LC were much lower: 2.60, 11.7, 6.13, and 0.25 μg kg^{-1}. The extracts all showed binding activities to oestrogen receptors α and β.

Ginger

Ginger root is widely used for digestive problems. There are several reports of finding aflatoxins in ginger powder (MacDonald and Castle 1996; Patel et al. 1996; Reddy et al. 2002). In India, OTA was found by ELISA in two of 25 samples of ginger at 23 and 80 μg kg^{-1} (Thirumala-Devi et al. 2001). There had not been any earlier report of finding mycotoxins in ginger supplements. The first author's laboratory analysed 25 bottles of ginger capsules (60 capsules/bottle, 625 mg/capsule) purchased from a botanical supplier. All of the samples contained aflatoxins and OTA at levels of 6.2–12.3 and 1.8–2.9 μg kg^{-1}, with averages of 8.7 and 2.2 μg kg^{-1}, respectively. The aflatoxin G_1 (AFG_1) level was about 10% higher than that of the AFB_1, which was unexpected since AFB_1 is usually the major aflatoxin found in most grains, nuts, and other agricultural commodities.

Capsicum

Capsicum is derived from the Greek word 'to bite'. Thus, *Capsicum* is known as the 'plant that bites back'. The genus includes chilli peppers, cayenne, pimento, paprika, red peppers, Tabasco peppers, and bell peppers and has been cultivated for thousands of years in tropical America, Africa, and India. There have been many reports of finding aflatoxins in capsicum. In Ethiopia, out of 60 samples each of ground red pepper and Shiro, eight (13%) and five (8.3%) were positive for aflatoxins, respectively (Fufa and Urga 1996). Only AFB_1 was detected in both types of foodstuff, ranging from 100 to 500 $\mu g\,kg^{-1}$ and from 250 to 525 $\mu g\,kg^{-1}$, respectively.

In India, samples of grades 1–3 chilli pod collected in 1998 and 1999 from the principal market yards and cold storage facilities were analysed for AFB_1 by an indirect competitive ELISA (Reddy et al. 2001). Of the 182 chilli samples 59% were contaminated with AFB_1 and 18% contained the toxin at non--permissible levels (>30 $\mu g\,kg^{-1}$). The highest AFB_1 concentration of 969 $\mu g\,kg^{-1}$ was found in one sample representing grade 3. Overall the greatest percentage of chilli pods showing AFB_1 levels higher than 30 $\mu g\,kg^{-1}$ was in grade 3. The lowest AFB_1 levels were found in chilli stored in cold facilities. Another study examined 100 chilli samples for OTA: 26 were found to contain over 10 $\mu g\,kg^{-1}$ OTA (Thirumala-Devi et al. 2000). In 12 samples the OTA concentration varied from 10 to 30 $\mu g\,kg^{-1}$, in ten samples from 30 to 50 $\mu g\,kg^{-1}$, in three samples from 50 to 100 $\mu g\,kg^{-1}$, and in one sample it was 120 $\mu g\,kg^{-1}$.

In Hungary, 91 spice samples (70 ground red pepper, six black pepper, five white pepper, five spice mix and five chilli samples) were analysed for aflatoxins and OTA by IAC/LC (Fazekas et al. 2005). Eighteen of the 70 ground red pepper samples contained AFB_1, and concentrations in seven of these ranged from 6.1 to 15.7 $\mu g\,kg^{-1}$. Thirty-two of the 70 ground red pepper samples contained OTA, eight of them in a concentration range of 10.6–66.2 $\mu g\,kg^{-1}$. One chilli sample was contaminated with OTA at 2.1 $\mu g\,kg^{-1}$.

Liquorice

Liquorice is derived from the ancient Greek words for 'sweet root'. Glycyrrhizin, a sweetener found in liquorice, is more than 50 times as sweet as sucrose and has pharmaceutical properties. The related Chinese liquorice (*G. uralensis*), which is used extensively in traditional Chinese medicine, contains this chemical in much greater concentration. Liquorice is a widely consumed herb and is the second most prescribed herb in China, after ginseng (Miller 1998). Several reports have been published on OTA in liquorice: three in Germany and one in Spain. In Germany, results of analysis of 83 liquorice roots and liquorice products used as food or supplements for medicinal purposes indicated that high levels of OTA were found in supplement products (0.3–64.3 $\mu g\,kg^{-1}$, median = 4.48 $\mu g\,kg^{-1}$) but not in children's tea (0.13–0.44 $\mu g\,kg^{-1}$) (Majerus et al. 2000). The peeled and unpeeled roots contained OTA averaging <0.1 and 4.3 $\mu g\,kg^{-1}$, respectively. The OTA levels (2.6–50.3 $\mu g\,kg^{-1}$) in medicinal products depended on the content of liquorice. Ten of 11 liquorice tablet samples were contaminated with OTA at 0.7–2.6 $\mu g\,kg^{-1}$ and the other liquorice products were at 0.46–46.2 $\mu g\,kg^{-1}$, with a median of 1.1 $\mu g\,kg^{-1}$. Nineteen samples of liquorice root and 19 liquorice sweets were analysed by LC/MS-MS (Bresch et al. 2000). Nine of the roots and 18 sweets contained OTA at levels of 0.3–217 and 0.5–3.0 $\mu g\,kg^{-1}$, respectively. Thirty samples of liquorice root, liquorice confectionery, liquorice block, and liquorice extract were analysed in Spain by LC and confirmed by methyl ester formation (Arino et al. 2007). All samples contained OTA. The 15 dry roots samples had an average OTA of 63.6 $\mu g\,kg^{-1}$ (1.4–253 $\mu g\,kg^{-1}$); the eight fresh roots averaged 9.2 $\mu g\,kg^{-1}$ (3.3–14.7 $\mu g\,kg^{-1}$); the four sweets averaged 3.8 $\mu g\,kg^{-1}$ (0.5–8.2 $\mu g\,kg^{-1}$); one liquid liquorice extract and one solid block contained 16 and 40 μg OTA kg^{-1}, respectively.

Red mould rice (RMR)

CIT has been found in red mould (yeast, or fermented) rice. RMR containing the cholesterol-lowering agent monacolin K is a bright reddish purple fermented rice, which acquires its colour from being cultivated with the mould *Monascus purpureus*. It is a commonly used red food colouring in East Asia and has also been used in Chinese herbal medicines. It is also sold as an over-the-counter dietary supplement for lowering cholesterol. However, in 1999 a study in the Netherlands detected the mycotoxin CIT in all the commercial *Monascus* samples at concentrations varying between 0.2 and 17.1 $mg\,kg^{-1}$ (Sabater-Vilar et al. 1999). In the USA, nine different commercially available RMR dietary supplements were tested and CIT was found in seven of them (Heber et al. 2001). In Taiwan, all *Monascus* products were found to contain CIT at concentrations of 0.28–6.29 $mg\,kg^{-1}$ (Liu et al. 2005). A mutant strain, *Monascus* sp. M12-69, was acquired by treatment with mutagenic agents of a wild strain M12 of *Monascus* screened from RMR samples gathered in China (Chen and Hu 2005). This strain can produce RMR

with a high concentration of monacolin K and low concentrations of CIT under optimum conditions.

Turmeric and kava-kava

There was one report in India of finding OTA in nine out of 25 turmeric sample at levels ranging from 11–102 $\mu g\,kg^{-1}$ (Thirumala-Devi et al. 2001). In the USA, 150 $\mu g\,kg^{-1}$ FB_1 was found in one of three powdered turmeric samples and OTA in three powdered kava-kava samples at levels of 0.3, 5.6, and 11.2 $\mu g\,kg^{-1}$ (Trucksess et al. 2006).

Methods of analysis for mycotoxins in botanicals

In general, the above limited data indicated that the levels of mycotoxins in botanicals are much lower than in grains and nuts. The results were based on traditional analytical methods with or without modifications. Commonly used methods are LC with FL detection, ELISA and occasionally TLC. Recently, LC-MS and LC-MS/MS techniques have also been applied.

Analytical methods for fumonisins

Fumonisins in medicinal plants were determined by methanol–water extraction, strong ion exchange solid-phase extraction column clean-up, *o*-phthaldialdehyde derivatization, reversed-phase LC separation, and FL detection (Martins et al. 2001; Omurtag and Yazicioğlu 2004). The LODs for the derivatization methods were 20–25 $\mu g\,kg^{-1}$. A recent study used a similar extraction solvent but an IAC was used for purification and isolation of FB_1 before LC-MS determination without derivatization (Sewram et al. 2006). The LOD was about 10 $\mu g\,kg^{-1}$.

Analytical methods for aflatoxins

The most common methods of analysis for aflatoxins include IAC clean-up, post-column bromination with electrochemical reaction cell (Kobra), or UV irradiation with a photochemical derivatization cell (PHRED), and LC-FL methods (Ali et al. 2005; Arranz et al. 2006; Romagnoli et al. 2007) or solid-phase extraction clean-up and LC-MS/MS (Ventura et al. 2004). One of these methods was tested in a mini-collaborative study by four laboratories for AFB_1 in senna pods (*Cassia angustifolia*), Devil's claw (*Harpagophytum procumbens*) and ginger roots (*Zingiber officinale*) (Arranz et al. 2006). The limit of quantitation (LOQ) was 1 $\mu g\,kg^{-1}$ and recoveries varied depending on the kind of herb. Another IAC/LC method had an LOQ of 0.05–0.1 $\mu g\,kg^{-1}$ (Gómez-Catalán et al. 2005). When applied to several herbs, recoveries of added aflatoxins were 50–60% for some of the herbs even though the reproducibility was good. The IAC procedure was modified by Ip and Che (2006) who replaced the phosphate-buffered saline (PBS) pH 7.4 with 0.1 M phosphate buffer pH 8.0 in order to improve recoveries of aflatoxins from certain Chinese highly acidic medicinal herbs. Three derivatization techniques to enhance the fluorescence of aflatoxin after IAC clean-up were compared: pre-column trifluoroacetic acid, post-column bromination (Kobra cell) and post-column UV irradiation (PHRED or UV cell) (Trucksess et al. 2006). Results of the three derivatization techniques were all comparable for ginseng, ginger, liquorice, and kava-kava. Recoveries of aflatoxin added to ginseng at levels from 2 to 16 $\mu g\,kg^{-1}$ were 80%. The LOD was about 1 $\mu g\,kg^{-1}$. Reif and Metzger (1995) developed an IAC/LC method for aflatoxins in medicinal herbs which had AFB_1 recoveries of 79–99% from valerian, fennel seed, and gourd seed. Aflatoxin in medicinal plants such as *Rhammus purshiana* was determined by LC-MS after methanol–water extraction and polymeric solid-phase clean-up (Ventura et al. 2004). A single-quadrupole MS using an electrospray ionization source operating in the positive-ion mode was used. Mean recoveries of added aflatoxins were about 77–110%. The LOD was 10 $\mu g\,kg^{-1}$ and the LOQ was 25 $\mu g\,kg^{-1}$. TLC methods were used in several older studies (Hitokoto et al. 1978; Trucksess and Stoloff 1980; Tanaka et al. 1988; Efuntoye 1999). There was no mention of LODs in most of these methods except that one method had an LOD of 1–5 $\mu g\,kg^{-1}$ (Tanaka et al. 1988). Non-specific reactions of antibodies to the sample matrix were often encountered when ELISA was used (Thirumala-Devi et al. 2000; Gray et al. 2004). This could result in over- or under-estimated toxin levels.

Ochratoxin A

LC with FL detection after extraction and purification is widely used for OTA in botanicals. Most of these methods are for liquorice because OTA is well known as a contaminant in liquorice roots and liquorice products. OTA in test samples was extracted by boiling in water for 10 min (Bresch et al. 2000). After the addition of sodium bicarbonate, adjustment of the pH to 7.4, and dilution with buffer, the diluted extract was purified on an IAC. LC-MS/MS turbo ion-spray ionization and multiple reaction monitoring were performed for the separation and quantitation of OTA in the samples. The LOQ of the method was about 0.3 $\mu g\,kg^{-1}$. Liquorice products for food,

supplements or medicinal purposes were extracted with sodium bicarbonate and methanol and the extract was passed through a phenyl cartridge then an IAC before LC-MS/MS analysis (Majerus et al. 2000). This method enabled the detection of OTA at levels $>0.3\,\mu g\,kg^{-1}$ in all products analysed. A method based on CEN (European Committee for Standardization) method EN 14132:2003 was applied to liquorice and derived products (Arino et al. 2007). The sample was extract with sodium bicarbonate–methanol, centrifuged, and passed through an IAC. LC-FD was used for separation and quantitation; the LOQ was $0.5\,\mu g\,kg^{-1}$ and recoveries were 91%.

A multi-toxin IAC column was recently used for aflatoxins and OTA in ginger and ginseng (Trucksess et al. 2007). This is the first report of using multi-toxin IAC for botanicals. After LC-FD separation and quantitation, recoveries of added aflatoxins and OTA were 70–80%. The LOD was about $0.1\,\mu g\,kg^{-1}$ and the LOQ was $1\,\mu g\,kg^{-1}$for AFB_1 and OTA. OTA contamination was found in powdered ginger and turmeric powder using an indirect competitive ELISA method (Thirumala-Devi et al. 2001). Samples were extracted with 0.5% potassium chloride in 70% methanol, then diluted before ELISA. The LOD and LOQ were 10 and $35\,ng\,kg^{-1}$, respectively.

Citrinin (CIT)

CIT was found in cultures inoculated with *Monascus* species (Blanc et al. 1995). An excellent review of analysis of CIT was published by Xu et al. (2006). The *Monascus* product known as RMR has been found to contain the cholesterol-lowering agent monacolin K (MK), including the lactone form (MKL) and the acid form (MKA), and CIT. High recovery rates for CIT, MKL and MKA are achieved by extracting the RMR with 95% ethanol at 60°C for 30 min, and separating the peaks of the three analytes using reversed-phase LC on a C-18 column, acetonitrile–water–trifluoroacetic acid (55 + 45 + 0.05, v/v/v) mobile phase, and UV and FL detectors (Lee et al. 2006). After extraction of CIT from *Monascus* cultures into acetonitrile–water (3 + 2, v/v), centrifugation and reverse-phase LC separation with FD detection at 334 nm, the recovery of added CIT was 97% (Xu et al. 2003b). A selective solvent extraction using toluene−ethyl acetate−formic acid was used to extract CIT from *Monascus* culture material providing red colorants for foods; recoveries were 87–126% at 1–2 $mg\,kg^{-1}$ spiking levels (Xu et al. 2003a). The LOD was $15\,mg\,kg^{-1}$ when an ELISA method was applied to determine CIT in Chinese RMR dietary supplements (Heber et al. 2001).

Zearalenone (ZON)

There have not been many studies on the analysis of botanicals for ZON. When ZON was found in extracts of ginseng roots (Gray et al. 2004), a commercially available ELISA kit and a published method (Ware et al. 1999) were used. There was no mention of LOD or LOQ.

Prevention and treatment of mycotoxins in botanicals

Surveys of fungal contamination in botanicals indicate many toxigenic moulds such *A. flavus*, *A. parasiticus*, and *Penicillium* spp. Although mycotoxins are not always detected in botanicals, under warm, humid conditions some moulds could produce aflatoxins and other toxins. Good manufacturing practice after harvesting botanicals such as cleaning, drying, and packaging will minimize mould growth and proliferation. Changing cultural practices such as wetting capsicum (chilli pods) by sprinkling with water before marketing will avoid conditions that favour mould growth and aflatoxin production (Reddy et al. 2001).

Microbiological decontamination of botanicals can be achieved by irradiation (Katušin-Ražem et al. 1983, 2001; Owczarczyk et al. 2000). However, some important measures and steps should be adopted (Xingwang and Jilan 1998). In order to have no significant biological or toxicological changes in traditional Chinese medicines after irradiation, proper conditions such as irradiating with 7 kGy for herbal medicine and with 5 kGy for some special herbal medicines are required. Herbs should be stored in a dry state and traditional Chinese medicines should be mixed with honey, forming a bolus to minimize decomposition.

Levels of OTA can be reduced by peeling the outer skin of liquorice roots (Majerus et al. 2000). It would be worthwhile to investigate whether this procedure is applicable for other roots to determine if OTA and AF levels in ginger, for example, can be lowered by this physical procedure.

Mycotoxins in dried fruit

Mycotoxins in dried vine fruit

Parallel to their occurrence in wine and grape juice resulting from contaminated grapes (Varga and Kozakiewicz 2006; Scott 2008), mycotoxins are often present in dried vine fruits. Surveys for OTA have been carried out in many countries, including Argentina, Canada, the Czech Republic, Finland, France, Germany, Greece, Hungary, Sweden, the

Netherlands, and the UK (Varga and Kozakiewicz 2006). Some maximum levels of OTA reported in the literature are $35\,\mu g\,g^{-1}$ in raisins analysed in Sweden (Möller and Nyberg 2003), $250\,\mu g\,kg^{-1}$ in raisins in Egypt (Youssef et al. 2000), $26\,\mu g\,kg^{-1}$ in sultanas analysed in Canada (Lombaert et al. 2004), $54\,\mu g\,kg^{-1}$ in unprocessed sultanas in Turkey (Meyvaci et al. 2005), $100\,\mu g\,kg^{-1}$ in processed Turkish sultanas (Aksoy et al. 2007), and $54\,\mu g\,kg^{-1}$ in currants analysed in the UK (MacDonald et al. 1999). Taking some of the data from four of these surveys as examples: Lombaert et al. (2004) found 79% of 85 samples of raisins and 59% of 66 samples of sultanas contained OTA above $0.1\,\mu g\,kg^{-1}$ with overall mean levels in all samples of $1.8\,\mu g\,kg^{-1}$ for both raisins and sultanas; Meyvaci et al. (2005) covered three production years for unprocessed sultanas in the Aegean region of Turkey, finding considerable variations in incidence and median levels with an overall mean level $3.4\,\mu g\,kg^{-1}$; while Aksoy et al. (2007) reported varying incidences over five years in *processed* Turkish sultanas (with a overall mean concentration $= 1.4\,\mu g\,kg^{-1}$). MacDonald et al. (1999) reported incidences of 95, 85, and 85% OTA $> 0.2\,\mu g\,kg^{-1}$ in 20 samples each of currants, raisins, and sultanas, respectively.

Aspergillus carbonarius was the predominant fungal source of OTA in dried vine fruits examined in Spain and Argentina (Abarca et al. 2003; Magnoli et al. 2004; Valero et al. 2005). Other OTA-producing fungi which have been isolated from grapes or dried vine fruit and are probable contributors to its presence in dried vine fruits include *A. niger* var. *niger* (Abarca et al. 2003; Magnoli et al. 2004; Romero et al. 2005) and *A. tubingensis* (Medina et al. 2005; Varga et al. 2006). In Greece, the highest frequency of OTA in currants and sultanas occurred in samples from sea level and the lowest occurred in samples from the highest altitudes (600–1000 m) (Pateraki et al. 2005). This parallels other findings that OTA concentrations in European Carignan grape musts increased the closer the vineyard was to the Mediterranean Sea (Roset 2003).

Sampling and subsampling of dried vine fruits for OTA is an important problem. Sample sizes that have been chosen for analysis are 500 g (Möller and Nyberg 2003; Lombaert et al. 2004), 1000 g (MacDonald et al. 1999) and 3000 g (Meyvaci et al. 2005). The heterogeneity of OTA in dried vine fruits is well illustrated by the study of Möller and Nyberg (2003), who prepared slurries of 150–275 g subsamples of raisins or currants in aqueous sodium bicarbonate. There were large differences between subsamples in some cases, e.g. 4.1/13.7, 0.9/4.5, 0.1/3.1, 0.2/34.6, 1.2/5.2 and 6.0/19.0 $\mu g\,kg^{-1}$.

In contrast to the large volume of work on OTA, there are fewer reports on the presence of aflatoxins in dried vine fruits. Maximum concentrations of AFB_1 found in raisins were $180\,\mu g\,kg^{-1}$ in India (Saxena and Mehrotra 1990), 550 and $300\,\mu g\,kg^{-1}$ in two studies in Egypt (Abdel-Sater and Saber 1999; Youssef et al. 2000), and $20\,\mu g\,kg^{-1}$ in imports to Greece (Apergi et al. 1998). Up to only $2\,\mu g\,kg^{-1}$ aflatoxins B_1 and B_2 were found in white sultanas analysed in Brazil (Iamanaka et al. 2007). Aflatoxigenic *A. flavus* has been isolated from grapes in Lebanon (El Khoury et al. 2006).

Mycotoxins in dried figs

Figs have also been found to contain aflatoxins and OTA as found in numerous surveys (Drusch and Ragab 2003; Drusch and Aumann 2005; Senyuva et al. 2007). Contamination of figs with aflatoxins begins during sun drying on the tree and continues during drying on the ground (Buchanan et al. 1975; Boyacioğlu and Gönül 1990; Özay and Alperden 1991). Levels can be very high: up to $76\,000\,\mu g\,kg^{-1}$ AB_1 (measured by TLC) (Steiner et al. 1993); up to $72\,\mu g\,kg^{-1}$ aflatoxin B_2 (AB_2) (Boyacioğlu and Gönül 1990); up to $180\,000\,\mu g\,kg^{-1}$ aflatoxin G_1 (AG_1) (measured by TLC) (Steiner et al. 1993); and up to $12\,300\,\mu g\,kg^{-1}$ OTA (by LC) (Steiner et al. 1993). A four-year survey of aflatoxins in figs intended for export from Turkey showed 2.6, 3.0, 5.1, and 2.7% incidences of total aflatoxins above $4\,\mu g\,kg^{-1}$ (Senyuva et al. 2007). Other mycotoxins — kojic acid (up to $8600\,\mu g\,kg^{-1}$) (Steiner et al. 1993) and patulin (up to $152\,\mu g\,kg^{-1}$) (Karaca and Nas 2006) — have been also found in dried figs as determined by GC-MS and LC, respectively.

More than one mycotoxin can occur in the same sample of figs: AFB_1 and OTA (Senyuva et al. 2005); AFB_1, AFG_1, kojic acid, and OTA (Steiner et al. 1993); and aflatoxins and patulin (Karaca and Nas 2006). In 99 samples of figs exported from Turkey only four contained both AFB_1 and OTA (Senyuva et al. 2005). In the study of Karaca and Nas (2006), total aflatoxin concentrations were significantly correlated with the patulin concentration in bright greenish-yellow (BGY) fluorescent figs from the Aegean region in Turkey. The little known species *Aspergillus alliaceus* may be responsible for the occasional contamination of figs in California with OTA (Doster et al. 1996, Bayman et al. 2002).

The sampling situation for aflatoxins in dried figs is similar to that in peanuts in that high levels of aflatoxins are associated with individual fruits. A large sample size is required, e.g. 20 kg from lots of 18–20 tonnes (Sharman et al. 1991, Hussain and Vojir 1993); 30 kg from 15–30 tonnes (European Commission (EC) 2006a); and 40 kg from 20 tonnes

(Bruland et al. 1992). Senyuva et al. (2007) found that three 10-kg subsamples were usually not uniformly contaminated. The sample may be slurried with water before taking subsamples for analysis (Sharman et al. 1991). For fig paste the sample size was 5 kg (Sharman et al. 1991).

More aflatoxin was found in BGY fluorescent figs (Steiner et al. 1988; Åkerstrand and Möller 1989). Thus, preliminary sorting by removing figs with BGY fluorescence can lower the aflatoxin contamination level of the lot. The AFB_1 level decreased from 23 to 0.3 $\mu g\,kg^{-1}$ by removal of all BGY fluorescent figs from a 56-kg sample (Steiner et al. 1988). However, absence of BGY fluorescence does not necessarily mean there are no aflatoxins in the sample (Hussain and Vojir 1993). In California figs, BGY fluorescence was not considered a promising screening tool (Doster and Michailides 1998). BGY fluorescence is more likely to be visible after cutting the fig open and this might be of use for figs used to make fig paste where figs are cut into quarters (Doster and Michailides 1998). BGY fluorescence cannot screen for OTA.

Mycotoxins in other dried fruit

Other dried fruits may also contain mycotoxins, particularly OTA and aflatoxin (Zohri and Abdel-Gawad 1993; Drusch and Ragab 2003; Drusch and Aumann 2005). The natural occurrence of OTA has been reported in dried apricots (Zohri and Abdel-Gawad 1993); dried plums (prunes) (Zohri and Abdel-Gawad 1993; Engel 2000; Iamanaka et al. 2005) and dates (Majerus et al. 1993, Abdel-Sater and Saber 1999; Miraglia and Brera 2002; Iamanaka et al. 2005). Dried slices of quince from markets in India contained up to 1630 $\mu g\,kg^{-1}$ of OTA (Sharma and Sumbali 1999). Co-occurrence of OTA and CIT in dry copra was reported from India (Kumari and Nusrath 1987).

AFB_1 has also been found in dried apricots (Apergi et al. 1998), prunes (Apergi et al. 1998), and dates (Abdel-Sater and Saber 1999; Herry and Lemetayer 1992). Dried pomegranate peel from India, which was analysed in the USA, contained 105 $\mu g\,kg^{-1}$ AFB_1 (Selim et al. 1996). The occurrence of aflatoxins in dry stored copra is well known and levels of up to 4000 $\mu g\ kg^{-1}$ AFB_1 have been reported (Samarajeewa and Arseculeratne 1983; Kumari et al. 1984). Saxena and Mehrotra (1990) found CIT as well as aflatoxins in dried coconut. ZON has been detected in dates in Egypt (Abdel-Sater and Saber 1999).

Maximum levels for mycotoxins in dried fruit

European regulations for certain mycotoxins in dried fruit have been published by the Commission of the European Communities. The maximum level for OTA in dried vine fruit (currants, raisins, sultanas) is 10 $\mu g\,kg^{-1}$ (EC 2006b). The maximum level for AFB_1 in dried fruit for human consumption or food ingredient is 2 $\mu g\,kg^{-1}$ and for total aflatoxins B_1, B_2, G_1, and G_2 is 4 $\mu g\,kg^{-1}$ (EC 2006b); for dried fruit to be subjected to sorting or other physical treatment the maximum levels are 5 $\mu g\,kg^{-1}$ AFB_1 and 10 $\mu g\,kg^{-1}$ aflatoxins B_1, B_2, G_1, and G_2. There are also sampling directives for dried figs and other dried fruits (EC 2006a). The European regulations for patulin (EC 2006b) have maximum limits of 25 $\mu g\,kg^{-1}$ patulin in solid apple products (which presumably would include dried apple rings) and 10 $\mu g\,kg^{-1}$ patulin in solid apple products for infants and young children.

Methods of analysis

Two methods of analysis for mycotoxins in dried fruit or processed dry fruit have been subjected to interlaboratory study. One was for the determination of OTA in currants, raisins, sultanas, dried figs, and mixed dried fruit (comprising dried pineapple, papaya, prunes, dates, and banana chips as well as sultanas) using extraction with acidic methanol, IAC clean-up and LC-FL. The method was tested in 24 laboratories at low $\mu g\,kg^{-1}$ levels (1.1–11.4 $\mu g\,kg^{-1}$) so that the European Union maximum level can be enforced (MacDonald et al. 2003). It should be noted that other workers (Möller and Nyberg 2003; Senyuva et al. 2005) have used a basic extraction with methanol–aqueous sodium bicarbonate, which gives better recoveries of OTA from figs and dried vine fruits than acid extraction. The second collaboratively studied method, also based on IAC/LC, was for the determination of aflatoxins in fig paste; it was studied in 16 laboratories with AFB_1 spiking levels of 1 and 4 $\mu g\,kg^{-1}$ and total aflatoxin spiking levels of 2.4 and 9.6 $\mu g\,kg^{-1}$ (Stroka et al. 2000). This method used post-column bromination. In a separate study electrochemical bromination and photochemical derivatization were found to be equally suitable for the analysis of fig paste for aflatoxins (Papadopoulou-Bouraoui and Anklam 2002).

LC-MS has been used to determine OTA in raisins (Buttinger et al. 2004; Lindenmeier et al. 2004) and aflatoxins in figs (Vahl and Jørgensen 1998).

Prevention of mycotoxins in dried fruit

As for the botanicals (see the section on 'Prevention and treatment of mycotoxins in botanicals'), exposure of humans to mycotoxins from consumption of dried fruits can be reduced (Drusch and Ragab 2003). The considerable research on

fungicides and vineyard management (soil cultivation, irrigation, and soil amendments) that has been carried out on grapes for the prevention of OTA in wine (Drouillard et al. 2003; Bellí et al. 2006; Leong et al. 2006) is applicable with regard to dried vine fruit. The code of sound vitivinicultural practices to minimize levels of OTA in vine-based products put forward by the Office International de la Vigne et du Vin (OIV) includes hygiene of the containers, measures to avoid fruit fly infestation, avoidance of overstacking, sorting, and drying conditions (Castellucci 2005). For the problem of aflatoxins in figs, it is important to have good techniques for orchard management, harvesting and drying, including decreasing *A. flavus* spores in the orchard, removal of damaged fruits and additional drying in a solar drier (Le Bars 1990; Özay et al. 1995). As indicated above, fluorescence sorting of dried figs can help prevent aflatoxin-contaminated figs from reaching the consumer (Steiner et al. 1988; Åkerstrand and Möller 1989). Irradiation of fresh fruits, including grapes and figs, can significantly decrease fungal counts (Aziz and Moussa 2002). Microflora can be eliminated after treatment of fruits with sulfur dioxide followed by drying; sodium and potassium metabisulfite were shown to reduce aflatoxins and mould counts in figs (Özay et al. 1995).

Conclusions

On basis of this review, it is clear that botanicals and dried fruits sold commercially can be contaminated with mycotoxins at levels exceeding regulations in some countries. It is essential to investigate further the presence of mycotoxins in these commodities. It is also essential to develop and apply strategies to prevent the formation of mycotoxins in them in order to ensure that botanicals and dried fruits are wholesome and safe for consumers.

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