

# Fungal Presence in Selected Tree Nuts and Dried Fruits



V.H. Tournas<sup>1</sup>, N.S. Niazi<sup>2</sup> and J.S. Kohn<sup>3</sup>

<sup>1</sup>Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA. <sup>2</sup>Georgetown University Medical School, Washington, D.C., USA. <sup>3</sup>Northeast Regional Laboratory, Food and Drug Administration, Jamaica, NY, USA.

**ABSTRACT:** Sixty-four tree nut samples (almonds, pecans, pine nuts, and walnuts) and 50 dried fruit samples (apricots, cranberries, papaya, pineapple, and raisins) were purchased from local supermarkets and analyzed for fungal contamination using conventional culture as well as molecular methods. The results of our study showed that the highest yeast and mold (YM) counts ( $5.34 \log_{10}$  CFU  $g^{-1}$ ) were found in walnuts and the lowest in pecans. The most common mold in nuts was *Aspergillus niger*, relatively low numbers of *A. flavus* were found across the board, while *Penicillium* spp. were very common in pine nuts and walnuts. Low levels ( $2.00$ – $2.84 \log_{10}$  CFU  $g^{-1}$ ) of yeasts were recovered from only two pine nut samples. Fungal contamination in dried fruits was minimal (ranging from  $<2.00$  to  $3.86 \log_{10}$  CFU  $g^{-1}$ ). The highest fungal levels were present in raisins. All papaya samples and the majority of cranberry, pineapple, and apricot samples were free of live fungi. The most common mold in dried fruits was *A. niger* followed by *Penicillium* spp. One apricot sample also contained low levels ( $2.00 \log_{10}$  CFU  $g^{-1}$ ) of yeasts.

**KEYWORDS:** dried fruits, tree nuts, fungal profiles

**CITATION:** Tournas et al. Fungal Presence in Selected Tree Nuts and Dried Fruits. *Microbiology Insights* 2015:8 1–6 doi:10.4137/MBI.S24308.

**RECEIVED:** January 23, 2015. **RESUBMITTED:** March 16, 2015. **ACCEPTED FOR PUBLICATION:** March 19, 2015.

**ACADEMIC EDITOR:** Raul Rivas, Editor in Chief

**TYPE:** Original Research

**FUNDING:** This work was supported solely by FDA funds. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

**COMPETING INTERESTS:** Authors disclose no potential conflicts of interest.

**COPYRIGHT:** © the authors, publisher and licensee Libertas Academica Limited. This is an open-access article distributed under the terms of the Creative Commons CC-BY-NC 3.0 License.

**CORRESPONDENCE:** valerie.tournas@fda.hhs.gov

Paper subject to independent expert blind peer review by minimum of two reviewers. All editorial decisions made by independent academic editor. Upon submission manuscript was subject to anti-plagiarism scanning. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties. This journal is a member of the Committee on Publication Ethics (COPE).

Published by Libertas Academica. Learn more about this journal.

## Introduction

Foods of plant origin, such as tree nuts and fruits, are known to foster the growth of various microorganisms including toxigenic and pathogenic fungal species. Tree nuts in particular have been often reported to contain potentially toxigenic molds such as *Aspergillus flavus*, *A. parasiticus*, *A. niger*, and other species capable of flourishing in this type of substrates. According to past research, peanuts and tree nuts (such as walnuts and pecans) were found to carry such organisms. Bayman et al<sup>1</sup> reported that the most common fungi in nuts were *Aspergillus*, *Rhizopus*, and *Penicillium*. *A. niger*, *A. flavus*, *A. parasiticus*, and *A. nomius* were reported from surveys conducted in the US, Europe, Australia, and South America.<sup>2–6</sup> The presence of various aspergilli and penicillia (eg, *A. niger*, *A. flavus*, *Penicillium crustosum*, etc) is of special concern since it has been established that some members of these species are capable of producing mycotoxins. *Aspergillus* section *Flavi* (*A. flavus*, *A. parasiticus*, and *A. nomius*) are known to produce the toxic and carcinogenic compounds aflatoxins (AFs) in foods and agricultural products including tree nuts, while black aspergilli (*Aspergillus* section *Nigri*) produce 145 secondary metabolites several of which are toxic to humans and animals. More specifically, *A. niger* could produce ochratoxin A (OTA) and fumonisin B<sub>2</sub> (FB<sub>2</sub>), while the majority of *A. carbonarius* strains produce OTA. Other possibly toxic metabolites often generated in large amounts by *Aspergillus* section *Nigri* are malformins, naphtho- $\gamma$ -pyrones and bicumarins

(kotanins).<sup>7</sup> Naturally occurring mycotoxins such as AFs, OTA, and FB<sub>2</sub> have also been reported from tree nuts. Low levels ( $<1.5$  ng  $g^{-1}$ ) of FB<sub>2</sub> were detected in a few samples of pick-out almonds.<sup>8</sup> AFs ( $15$ – $25$  ng  $g^{-1}$ ) and zearalenone (ZEA) ( $125$  ng  $g^{-1}$ ) were found in walnuts.<sup>9</sup> Higher levels of AFs (up to  $95$  ng  $g^{-1}$ ) were reported in almonds,<sup>6,10</sup> while sterigmatocystin (ST) was detected in in-shell pecans.<sup>11</sup>

Fruits after harvest carry plant pathogenic species from the field, which may or may not survive the drying process. Live potentially toxigenic storage fungi were found in various dried fruits in the past. Tournas (2004, unpublished data) isolated *A. niger*, *A. carbonarius*, and *A. ochraceus* from raisins, while Hocking et al<sup>12</sup> reported the presence of *Aspergillus* section *Nigri* and *A. ochraceus* in grapes and grape-derived products such as raisins. The presence of OTA in dried fruits such as currants, raisins, and sultanas has also been reported in the literature.<sup>13,14</sup> If the moisture of the dried products is kept at the proper levels, they should not support mold growth. When their water activity ( $a_w$ ), however, surpasses 0.65, they become prone to spoilage by some postharvest fungal pathogens including xerophilic aspergilli and eurotia depending on the  $a_w$  mark.<sup>15</sup> The presence of such species could compromise the quality of the affected products and be the cause of serious health concerns for the consumers. Although reports on fungal profiles of tree nuts and dried fruits exist in the literature, the ongoing changes in fungicide types and levels used may have over time caused changes in mycobiota present on these



commodities. Therefore, we deemed it appropriate to revisit the issue and conducted this study in order to evaluate the mycological quality of various tree nuts and dried fruits presently available in the U.S. market.

## Materials and Methods

**Materials.** Dried fruits and tree nuts tested in this study were obtained either in their unopened packages or from bulk jars and trays (1-lb portions) from four supermarket chains (eight stores) in the Washington, D.C. area; each sample was drawn from an individual lot. All samples were kept at room temperature from the time of purchase until commencement of analysis, which was conducted within 48 hours from the time of purchase. Fifty grams from each sample was analyzed for fungal contamination.

**Chemicals, reagents, and other supplies.** Polymerase chain reaction (PCR) reagents and DNA ladder were purchased from Fisher Scientific. DNA extraction kits were obtained from Norgen Biotek Corporation; primers were purchased from Integrated DNA Technologies (IDT). Agarose gels were obtained from Bio-Rad. All mycological media utilized for the isolation and conventional plating identification of fungal specimens were prepared in-house according to the methods and formulas described by Pitt and Hocking<sup>15</sup> and in the *Bacteriological Analytical Manual*.<sup>16</sup>

**Isolation and quantification of molds and yeasts.** Samples were tested as follows: Fifty grams of each product was aseptically transferred into sterile blender jars. Subsequently, they were blended in 450 mL of 0.1% peptone for 45 seconds. Serial dilutions of the homogenates in 0.1% peptone were surface-plated on duplicate DG18 agar (0.1 mL/plate), and the plates were incubated for 5 days at 25°C. Then, colonies were counted, and counts were reported as colony-forming units per gram (CFU g<sup>-1</sup>).

**Speciation of isolated fungal strains.** The recovered isolates were purified by reculturing on potato dextrose agar (PDA) (DIFCO) plates and identified to genus or species level using the conventional methods and keys described in *Identification of Common Aspergillus Species, A Laboratory Guide to Common Penicillium Species, Fusarium Species: An Illustrated Manual for Identification*, and *Fungi and Food Spoilage*.<sup>15,17–19</sup> Isolates that could not be speciated by conventional plating methods, were identified using molecular techniques as described below.

**Molecular method—DNA extraction.** Fungal DNA was isolated as follows: The fungal strains recovered from tree nuts and dried fruits were grown on solid media at 25°C for 5 days; then a small culture portion from each isolate was transferred to a 15-mL conical tube containing potato dextrose broth and incubated at 30°C for 24 hours. After the incubation period, the cultures were centrifuged for 10 minutes at 10,000 rpm to pellet, and the supernatants were discarded. Subsequently, 10 mL of phosphate buffered saline (PBS) buffer was added to each tube and the pellets were homogenized

by vortexing. The tubes were centrifuged again under the same conditions as above, and the supernatants were discarded. Then, 1 mL of PBS buffer was added to each sample and the tubes were vortexed. Subsequently, 50 µl was removed from each tube and transferred to 2.0-mL microcentrifuge tubes to proceed with DNA extraction. DNA extraction was carried out using the Norgen Biotek Fungi/Yeast Genomic DNA Isolation Kit according to manufacturer's instructions.

**Polymerase chain reaction.** The primers encoding the  $\beta$ -tubulin gene, Bt2a (5'-GTAACCAAATCGGTGCTGC TTTC-3') and Bt2b (5'-ACCCTCAGTGTAGTGACCC TTGGC-3') were used.<sup>20</sup> The method described by Asefa et al<sup>21</sup> with minor modifications was utilized. PCR was performed in reaction tubes with a final volume of 50 µL consisting of 25 µL Promega GoTaq Hot Start colorless master mix, 21.5 µL Promega nuclease-free water, 1.5 µL DNA template, and 1 µL of each primer. The mixture was spun, and the PCR reaction was run in an Eppendorf 2231 Thermocycler (Eppendorf North America) programmed with the following conditions: Denaturation at 95°C for 10 minutes, 38 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes.

**Gel electrophoresis.** The PCR products were subjected to electrophoresis in 1% agarose gels (Bio-Rad ReadyAgarose ethidium bromide precast gels) to determine the band sizes and to confirm amplification of the DNA. Gels were run using Tris-borate-EDTA (TBE) buffer at 60 V for 70 minutes, and were subsequently visualized and photographed under UV light in an Alpha Imager (Alpha Innotech Corp.).

**Sequencing and species identification.** Purification and sequencing of the PCR products was performed by MCLAB, and the resulted sequences were trimmed and edited using FinchTV 1.4.0 software. Subsequently, sequence comparisons and speciation were done by utilizing the basic local alignment search tool (BLAST) in Genbank ([www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST)).

## Results

**Fungal contamination in tree nuts.** In this study, 64 tree nut samples (consisting of pecans, almonds, pine nuts, and walnuts) were tested for the presence and levels of fungal contaminants. All walnut, 91% of pine nut, and 76% of almond samples carried live fungi. Mold incidence was very low (6%) in pecans. The highest yeast and mold (YM) counts (5.34 log<sub>10</sub> CFU g<sup>-1</sup>) were recovered from walnuts, and the lowest levels (2.00 log<sub>10</sub> CFU g<sup>-1</sup>) were found in pecans (Table 1).

A total of 117 fungal isolates including potentially toxigenic *Aspergillus*, *Eurotium*, *Penicillium*, *Fusarium*, and *Alternaria* species were recovered during this study (Table 2). The most common organisms were aspergilli (found in 66% of the samples and comprising 46% of the isolates); these organisms were present in all commodities tested. Black aspergilli (*A. niger* and *A. tubingensis*) were the most frequent contaminants of

**Table 1.** Fungal contamination levels in various tree nuts and dried fruits.

PRODUCT	NUMBER OF SAMPLES TESTED	YM COUNTS RANGE ( $\log_{10}$ CFU $g^{-1}$ )	FREQUENCY*
<b>Tree nuts</b>			
Almonds	17	<2.00–4.00	76
Pecans	16	<2.00–2.00	6
Pine nuts	11	<2.00–3.53	91
Walnuts	20	2.65–5.34	100
<b>Dried fruits</b>			
Apricots	8	<2.00–2.60	25
Cranberries	10	<2.00–2.30	20
Papaya	10	<2.00	0
Pineapple	12	<2.00–2.00	8
Raisins	10	<2.00–3.86	60

Note: \*Frequency is the percentage of contaminated samples.  
Abbreviations: YM, yeast and mold; CFU, colony forming units.

tree nuts. *A. niger* was found in 35% almond, 36% pine nut, and 30% walnut samples at levels sometimes exceeding 3.00  $\log_{10}$  CFU  $g^{-1}$ , while *A. tubingensis* was isolated from almonds and walnuts at high frequency (47% and 60% of the samples, respectively) and sometimes reached levels higher than 4.00  $\log_{10}$  CFU  $g^{-1}$ . *A. flavus* was detected in all tree nut commodities at relatively low levels. This organism was found in 17% of the overall samples, but only one pine nut sample carried >3.00  $\log_{10}$  CFU  $g^{-1}$ . The pathogenic species *A. fumigatus* was also recovered at low levels (2.30  $\log_{10}$  CFU  $g^{-1}$ ) from one pine nut and one walnut sample. *Alternaria* species were found in all tree nut types tested in this study except in pecans, but their levels did not reach 3.00  $\log_{10}$  CFU  $g^{-1}$ . A number of penicillia including the potentially toxigenic species *P. crustosum*, *P. brevicompactum*, and *P. polonicum* were also isolated from walnuts and/or pine nuts at levels exceeding 3.00  $\log_{10}$  CFU  $g^{-1}$ . One walnut sample contained elevated levels (4.26  $\log_{10}$  CFU  $g^{-1}$ ) of *P. brevicompactum*, while two other samples of the same commodity carried above 4.00  $\log_{10}$  CFU  $g^{-1}$  of *P. crustosum*. Eurotia were generally recovered at low levels and frequency during this study. The same was true for *Fusarium* spp; *Fusarium* levels reached 3.00  $\log_{10}$  CFU  $g^{-1}$  in only one walnut sample. Other fungal contaminants recovered from tree nuts at low levels and frequencies were *Cladosporium* spp., *Rhizopus* spp., and yeasts (Table 2).

**Fungal contaminants in dried fruits.** The results of the analysis of dry fruits are shown in Tables 1 and 3. Of the 50 dried fruit samples tested, only 25% of the apricots, 20% of the cranberries, 8% of the pineapple, and 60% of the raisins were contaminated with live fungi. No live molds or yeasts were isolated from papaya. The YM levels in the first three commodities were low (2.60  $\log_{10}$  CFU  $g^{-1}$  or lower); raisins generally carried higher YM counts, reaching 3.86

$\log_{10}$  CFU  $g^{-1}$  (Table 1). The main contaminant of raisins was *A. niger*, found in 50% of the samples, while *A. tubingensis* was recovered from pineapple and low levels of penicillia were isolated from apricots and cranberries (Table 3).

## Discussion

In this study, the mycological quality of various tree nuts and dried fruits sold in U.S. retail was examined. Tree nuts were found to contain sometimes high viable counts of potentially toxigenic molds such as *A. niger*, *A. tubingensis*, and *Penicillium* spp. *A. flavus* and *Fusarium* spp. were also encountered but at a lower rate. Past studies have shown similar contamination profiles of these commodities. According to Jimenez et al,<sup>10</sup> penicillia, aspergilli, and *Rhizopus* spp. were the most common molds found in almonds and other nuts. *A. flavus*, *A. niger*, as well as *Penicillium* and *Rhizopus* spp. were isolated from 76%, 71%, 86% and 50% of almond samples, respectively. Some of these isolates were capable of producing one or more of the mycotoxins: AFs (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, AFG<sub>2</sub>), OTA, patulin (PAT), ST, citrinin (CIT), ZEA, penicillic acid, and griseofulvin. Palumbo and O'Keefe,<sup>8</sup> after testing inedible pick-out almonds for fungal contamination, found that *Aspergillus* section *Nigri* members (eg, *A. tubingensis*, *A. niger*, and *A. awamori*) were frequent contaminants. *A. niger* and *A. awamori* comprised 27% of all mold strains recovered. Seventy-two percent of these strains produced FB<sub>2</sub> on CY20S agar, and 19% produced OTA. Kenjo et al<sup>22</sup> reported the presence of *Aspergillus* sections *Nigri* and *Flavi* (*A. niger*, *A. flavus*, *A. nomius*, *A. parasiticus*, and *A. parvisclerotigenus*) as well as *Penicillium*, *Cladosporium*, and *Rhizopus* spp. in commercial almond powder. Total fungal counts ranged between <2.00 and 3.93  $\log_{10}$  CFU  $g^{-1}$ . AFB<sub>1</sub> and AFB<sub>2</sub> were produced by some *A. flavus* and *A. parvisclerotigenus* strains, while AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub> were elaborated by all *A. nomius* and *A. parasiticus* isolates. Bayman et al,<sup>1</sup> after testing 1100 tree nut samples (including almonds, pistachios, walnuts, and Brazil nuts) without visible insect damage, also reported that the most common molds in these commodities were *Aspergillus*, *Penicillium*, and *Rhizopus*. Harvest and postharvest practices seemed to affect the nut mycoflora. Rodrigues et al<sup>6</sup> tested almonds for the presence of *Aspergillus* section *Flavi* molds. These investigators reported that *A. parasiticus* was the most frequent, comprising 56% of the isolates, followed by *A. flavus* (36% of isolates) and *Aspergillus tamaritii* (8% of isolates). Twenty-eight percent of *A. flavus* strains produced AFBs, while 100% of *A. parasiticus* isolates elaborated AFBs and AFGs.

Our study revealed the presence of the potentially toxigenic molds *A. niger*, *A. flavus*, *Penicillium* spp. and *P. polonicum* in pine nuts at levels higher than 3.00  $\log_{10}$  CFU  $g^{-1}$ . Fungal contamination of pine nuts has also been reported in past studies. Weidenborner<sup>23</sup> determined that the dominant fungal contaminants of pine nuts were *Cladosporium* (37% of the isolates) followed by *Phoma* (19% of the isolates), and that 16 of the 31 isolated species (including *Alternaria alternata*, *Aspergillus versicolor*, *A. niger*,

**Table 2.** Fungal species and levels recovered from selected tree nuts.

ORGANISM	CONTAMINATION LEVELS ( $\log_{10}$ CFU $g^{-1}$ – RANGE)			
	ALMONDS ( $n = 17$ )	PECANS ( $n = 16$ )	PINE NUTS ( $n = 11$ )	WALNUTS ( $n = 20$ )
<i>Alternaria</i> spp.	<2.00–2.84 (12)	ND	<2.00–2.60 (9)	<2.00–2.30 (10)
<i>Aspergillus</i> spp.	<2.00–2.00 (6)	ND	<2.00–2.70 (9)	<2.00–2.48 (5)
<i>Aspergillus flavus</i>	<2.00–2.48 (18)	<2.00–2.00 (6)	<2.00–3.36 (27)	<2.00–2.84 (20)
<i>Aspergillus niger</i>	<2.00–3.00 (35)	ND	<2.00–3.08 (36)	<2.00–3.28 (30)
<i>Aspergillus fumigatus</i>	ND	ND	<2.00–2.30 (9)	<2.00–2.30 (5)
<i>Aspergillus parasiticus</i>	ND	ND	ND	<2.00–2.00 (5)
<i>Aspergillus tubingensis</i>	<2.00–4.00 (47)	ND	ND	<2.00–5.34 (60)
<i>Aspergillus versicolor</i>	<2.00–2.00 (6)	ND	ND	ND
<i>Aureobasidium pullulans</i>	ND	ND	<2.00–2.18 (9)	ND
<i>Cladosporium</i> spp.	<2.00–2.30 (6)	ND	ND	<2.00–2.48 (5)
<i>Diplodia</i> spp.	ND	ND	<2.00–2.30 (9)	ND
<i>Eurotium</i> spp.	ND	ND	<2.00–2.30 (9)	<2.00–2.95 (10)
<i>Eurotium chevalieri</i>	ND	ND	<2.00–2.48 (9)	ND
<i>Fusarium</i> spp.	<2.00–2.18 (12)	ND	ND	<2.00–3.00 (10)
<i>Fusarium musae</i>	ND	ND	ND	<2.00–2.00 (5)
<i>Penicillium</i> spp.	ND	ND	<2.00–3.04 (36)	<2.00–2.95 (15)
<i>Penicillium brevicompactum</i>	ND	ND	ND	<2.00–4.26 (15)
<i>Penicillium crustosum</i>	<2.00–2.90 (6)	ND	ND	<2.00–4.29 (35)
<i>Penicillium echinulatum</i>	ND	ND	<2.00–3.00 (9)	ND
<i>Penicillium georgiense</i>	ND	ND	ND	<2.00–2.00 (5)
<i>Penicillium glabrum</i>	ND	ND	<2.00–2.30 (9)	<2.00–3.18 (5)
<i>Penicillium hispanicum</i>	ND	ND	ND	<2.00–3.00 (5)
<i>Penicillium polonicum</i>	ND	ND	<2.00–3.40 (19)	ND
<i>Penicillium solitum</i>	ND	ND	ND	<2.00–4.04 (10)
<i>Rhizopus</i> spp.	ND	ND	ND	<2.00–3.30 (5)
Yeasts	ND	ND	<2.00–2.84 (18)	ND
No growth	(24)	(94)	(9)	(0)

**Note:** Numbers in parentheses indicate % samples contaminated with respective organisms.

**Abbreviations:** CFU, colony forming units;  $n$ , number of samples tested; ND, not detected.

**Table 3.** Fungal species and levels recovered from various dried fruits.

ORGANISM	CONTAMINATION LEVELS ( $\log_{10}$ CFU $g^{-1}$ – RANGE)				
	APRICOTS ( $n = 8$ )	CRANBERRIES ( $n = 10$ )	PAPAYA ( $n = 10$ )	PINEAPPLE ( $n = 12$ )	RAISINS ( $n = 10$ )
<i>Aspergillus niger</i>	ND	ND	ND	ND	<2.00–3.86 (50)
<i>Aspergillus tubingensis</i>	ND	ND	ND	<2.00–2.00 (8)	ND
<i>Cladosporium</i> spp.	ND	ND	ND	ND	<2.00–2.30 (10)
<i>Penicillium</i> spp.	<2.0–2.60 (12)	<2.0–2.00 (10)	ND	ND	ND
<i>Penicillium palitans</i>	ND	<2.00–2.30 (10)	ND	ND	ND
Yeasts	<2.00–2.00 (12)	ND	ND	ND	ND
No growth	(75)	(80)	(100)	(92)	(40)

**Note:** Numbers in parentheses indicate % samples contaminated with respective organisms.

**Abbreviations:** CFU, colony forming units;  $n$ , number of samples tested; ND, not detected.



*Eurotium chevalieri*, *Penicillium aurantiogriseum*, *P. viridicatum*, *P. citrinum*, *P. crustosum*, *P. puberulum*, *P. expansum*, *P. glabrum*, and *Trichothecium roseum*) were potentially toxigenic. Marin et al,<sup>24</sup> on the other hand, reported the presence of *Fusarium proliferatum* in pine nuts. Fifty-four percent of the *F. proliferatum* strains isolated by these investigators produced fumonisin B<sub>1</sub> (FB<sub>1</sub>) in shelled pine nuts, while 18% elaborated the same toxin in whole pine nuts in a laboratory study. During our study, no live fusaria were found in this commodity.

According to the results of our study, the most frequent molds found in walnuts were penicillia (including *P. brevicompactum*, *P. crustosum*, *P. solitum*, *P. glabrum*, and *P. hispanicum*), *A. tubingensis*, *A. niger*, and *A. flavus*. *Fusarium* spp. were isolated from only 3 out of 20 samples. Past research on fungal contamination of walnuts has shown similar microfungi profiles. Abdel-Hafez and Saber<sup>9</sup> reported that *A. flavus*, *A. niger*, *A. fumigatus*, *Cladosporium* spp., *Penicillium chrysogenum*, and *P. oxalicum* were the most frequent contaminants, while *Fusarium verticillioides*, *F. equiseti*, and *F. oxysporum* were less common in walnuts in Egypt. Bacchetti and Arp,<sup>25</sup> on the other hand, reported the isolation of a *P. crustosum* strain from moldy walnuts; this isolate was able to produce penitrem A toxin.

In our study, pecans had minimal fungal contamination. This is in contrast with past studies that reported the presence of various mold contaminants in pecans with potential to produce mycotoxins.<sup>11,26</sup> Schroeder and Hein<sup>11</sup> demonstrated the presence of *A. flavus* (found in 43% of the samples) and *A. glaucus* (encountered in 35% of tested samples) in in-shell pecans; these organisms are known to produce ST. Trucksess et al,<sup>26</sup> on the other hand, reported that some *A. flavus* and *A. tamarii* strains isolated from pecans were capable of producing cyclopiazonic acid (CPA). Propylene oxide (PO) or other fungicidal treatment(s) applied to pecans after harvest may be responsible for the low YM numbers found in our study. Blanchard and Hanlin<sup>27</sup> demonstrated that PO application caused the destruction of 80%–92% of surface microorganisms.

Among the dried fruits tested during our experiment, only raisins showed a notable degree of fungal invasion, mainly by *A. niger*. Past studies also showed similar mold profiles in raisins. Iamanaka et al<sup>28</sup> reported that raisins (black and white sultanas) were contaminated with potentially toxigenic molds. The most frequently isolated fungi from that study were *A. niger*, followed by *A. ochraceus* and *A. carbonarius*. The presence of this group of fungi is troublesome because they are capable of producing mycotoxins in vine fruits. In general, members of the *A. niger* aggregate (eg, *A. niger* and *A. tubingensis*) were found to be more prevalent on grapes than *A. carbonarius* but a much higher percentage of the *A. carbonarius* strains were OTA producers.<sup>29–31</sup> According to Iamanaka et al,<sup>28</sup> 15% of *A. niger*, 60% of *A. carbonarius*, and 87% of *A. ochraceus* strains isolated from black and white sultanas were able to produce OTA. The latter study also revealed the presence

of OTA in 33% of the black sultana samples at levels above 5 ng g<sup>-1</sup>. OTA-producing *A. niger* var. *niger* and *A. tubingensis* were also isolated from dried vine fruits or grapes by other investigators,<sup>3,32–34</sup> while aflatoxin-producing *A. flavus* was isolated from grapes in Lebanon.<sup>35</sup> According to our findings, a small percentage of the dried apricot and cranberry samples contained very low levels of penicillia, while papaya was free of live fungi. Iamanaka et al<sup>28</sup> also reported the absence of live molds from some dried fruits including apricots. Scarcity or absence of live fungi from these commodities perhaps was due to a postharvest microbe-destructing treatment.<sup>36</sup>

## Conclusions

From the results of our study and past literature, it is obvious that mold and mycotoxin contamination of tree nuts and raisins is an ongoing problem. Measures to reduce fungal spore numbers carried on the vine fruits and nuts from the orchard as well as adherence to good manufacturing practices (GMPs) during and after harvest and quick drying are essential for the prevention or minimization of mold growth and mycotoxin contamination in the dried products. Additionally, in order to preserve the quality of these commodities, the products should be kept under conditions that ensure maintenance of appropriate moisture levels throughout storage and marketing. Since nuts contain very low levels of soluble carbohydrates, a small increase in moisture content (eg, condensation due to temperature changes during transport and storage) can result in substantial increase of  $a_w$ . Increase of  $a_w$  above a certain level will promote the growth of some fungal species, which can cause spoilage and possibly produce mycotoxins.

Low mold contamination in pecans and some dried fruits perhaps reflects the use of some kind of fungicidal treatment after harvest and before storage and marketing (eg, PO or heat application). If such mold-inactivating treatment was applied, any mycotoxins or spoilage byproducts formed before this treatment most likely would still be active and hazardous to human health. Therefore, future studies should be directed toward testing and establishing mycological profiles of these commodities using samples collected prior to application of mold-eradicating processing.

## Acknowledgments

The authors are grateful to Eugenia Katsoudas (Office of Regulatory Affairs/FDA) and Carol Weaver (Center for Food Safety and Applied Nutrition/FDA) for their technical assistance during the preparation of the manuscript.

## Author Contributions

Performed experiments: VHT, NSN. Planned the experiments and wrote and edited the manuscript: VHT. Assisted with editing the manuscript: JSK. All authors contributed to discussion of the results and reviewed and approved the final version of the manuscript.



## REFERENCES

1. Bayman P, Baker JL, Mahoney NE. *Aspergillus* on tree nuts: incidence and associations. *Mycopathologia*. 2002;155:161–169.
2. Jorgensen K. Occurrence of ochratoxin A in commodities and processed food—a review of EU occurrence data. *Food Addit Contam*. 2005;22(suppl 1):26–30.
3. Magnoli C, Astoreca A, Ponsone L, et al. Survey of mycoflora and ochratoxin A in dried vine fruits from Argentina markets. *Lett Appl Microbiol*. 2004;39:326–331.
4. Pitt JI, Hocking AD, Bhudhasamai K, Miscamble BF, Wheeler KA, Tanboon-Ek P. The normal mycoflora of commodities from Thailand. 1. Nuts and oilseeds. *Int J Food Microbiol*. 1993;20:211–226.
5. Richard JL, Payne GA. *Mycotoxins: Risks in Plant and Animal, and Human Systems*. Raleigh: CAST Task Force Report; 2003.
6. Rodrigues P, Venancio A, Lima N. Aflatoxicogenic fungi and aflatoxins in Portuguese almonds. *Sci World J*. 2012;47:1–9.
7. Nielsen KF, Mogensen JM, Johansen M, Larsen TO, Frisvad JC. Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Anal Bioanal Chem*. 2009;395:1225–1242.
8. Palumbo JD, O'Keefe TL. Distribution and mycotoxigenic potential of *Aspergillus* section *Nigri* species in contaminated almonds. *J Food Prot*. 2013;76:702–706.
9. Abdel-Hafez AI, Saber SM. Mycoflora and mycotoxins of hazelnut (*Corylus avellana* L.) and walnut (*Juglans regia* L.) seeds in Egypt. *Zentralbl Mikrobiol*. 1993;148:137–147.
10. Jimenez M, Mateo R, Querol A, Huerta T, Hernandez E. Mycotoxins and mycotoxigenic molds in nuts and sunflower seeds for human consumption. *Mycopathologia*. 1991;115:121–127.
11. Schroeder HW, Hein H Jr. Natural occurrence of sterigmatocystin in in-shell pecans. *Can J Microbiol*. 1977;23:639–641.
12. Hocking AD, Leong SL, Kazi BA, Emmett RW, Scott ES. Fungi and mycotoxins in vineyards and grape products. *Int J Food Microbiol*. 2007;119:84–88.
13. Trucksess MW, Scott PM. Mycotoxins in botanicals and dried fruits: a review. *Food Addit Contam*. 2008;25:181–192.
14. Moller TE, Nyberg M. Ochratoxin A in raisins and currants: basic extraction procedure used in two small marketing surveys of the occurrence and control of the heterogeneity of the toxins in samples. *Food Addit Contam*. 2003;20:1072–1076.
15. Pitt JI, Hocking AD. *Fungi and Food Spoilage* [e-book]. Weimar, Texas, USA: Culinary and Hospitality Industry Publications Services; 2009. Available at: <http://www.chipsbooks.com/fungi.htm>. Accessed November 22, 2014.
16. BAM Online. *Bacteriological Analytical Manual* [e-book]. Gaithersburg, Maryland: AOAC International; 2001. Available at: <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>. Accessed December 10, 2014.
17. Klich MA. *Identification of Common Aspergillus Species*. Utrecht: Centraalbureau voor Schimmelcultures; 2002.
18. Pitt JI. *A Laboratory Guide to Common Penicillium Species*. North Ryde, N.S.W: Commonwealth Scientific and Industrial Research Organization; 1985.
19. Nelson PE, Toussoun TA, Marasas WFO. *Fusarium Species: An Illustrated Manual for Identification*. University Park, Pennsylvania: The Pennsylvania State University Press; 1983.
20. Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Appl Environ Microbiol*. 1995;61:1323–1330.
21. Asefa DT, Gjerde RO, Sidhu MS, et al. Mold contaminants on Norwegian dry-cured meat products. *Int J Food Microbiol*. 2009;128:435–439.
22. Kenjo T, Ishide Y, Aoyama K, Ichinoe M. Fungal population and distribution of aflatoxicogenic fungi in commercial almond powder products. *Shokubin Eiseigaku Zasshi*. 2007;48:90–96.
23. Weidenborner M. Pine nuts: the mycobiota and potential mycotoxins. *Can J Microbiol*. 2001;47:460–463.
24. Marin S, Ramos AJ, Vazquez C, Sanchis V. Contamination of pine nuts by fumonisin produced by strains of *Fusarium proliferatum* isolated from *Pinus pinea*. *Lett Appl Microbiol*. 2007;44:68–72.
25. Bacchetti P, Arp LH. Moldy walnut toxicosis of a dog caused by the mycotoxin, penitrem A. *Mycopathologia*. 1981;76:55–58.
26. Trucksess MW, Mislivec PB, Young K, Bruce VR, Page SW. Cyclopiazonic acid production by cultures of *Aspergillus* and *Penicillium* species isolated from dried beans, corn meal, macaroni and pecans. *JAOAC Int*. 1987;70:123–126.
27. Blanchard RO, Hanlin RT. Effect of propylene oxide treatment on the microflora of pecans. *Appl Microbiol*. 1973;26:768–772.
28. Iamanaka BT, Taniwaki MH, Menezes HC, Vicente E, Fungaro MH. Incidence of toxigenic fungi and ochratoxin A in dried fruits sold in Brazil. *Food Addit Contam*. 2005;22:1258–1263.
29. Battilani P, Giorni P, Bertuzzi T, Formenti S, Pietri A. Black aspergilli and ochratoxin A in grapes in Italy. *Int J Food Microbiol*. 2006;111:S53–S60.
30. Perrone G, Mule G, Susca A, Battilani P, Pietri A, Logrieco A. Ochratoxin A production and amplified fragment length polymorphism analysis of *Aspergillus carbonarius*, *Aspergillus tubingensis* and *Aspergillus niger* strains isolated from grapes in Italy. *Appl Environ Microbiol*. 2006;72:680–685.
31. Perrone G, Susca A, Cozzi G, et al. Biodiversity of *Aspergillus* species in some important agricultural products. *Stud Mycol*. 2007;59:53–66.
32. Medina A, Mateo R, Lopez-Ocana L, Valle-Algarra FM, Mateo F, Jimenez M. Study of Spanish grape mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* section *Nigri*. *Appl Environ Microbiol*. 2005;71:4696–4702.
33. Romero SM, Comerio RM, Larumbe G, Ritieni A, Vaamonde G, Fernandez Pinto V. Toxigenic fungi isolated from dried vine fruits in Argentina. *Int J Food Microbiol*. 2005;104:43–49.
34. Varga J, Kocsube S, Koncz Z, Teren J. Mycobiota and ochratoxin A in raisins purchased in Hungary. *Acta Aliment*. 2006;35:289–294.
35. El Khoury A, Rizk T, Lteif R, Azouri H, Delia M-L, Lebrihi A. Occurrence of ochratoxin A- and aflatoxin B1-producing fungi in Lebanese grapes and ochratoxin A content in musts and finished wines during 2004. *J Agric Food Chem*. 2006;54:8977–8982.
36. Kendall P, Sofos J. *Drying Fruits*. *Food and Nutrition Series/Preparation*: Colorado State University Extension; 2014. Available at: <http://www.ext.colostate.edu/pubs/foodnut/09309.html>. Accessed March 5, 2015. [Fact Sheet No. 9.309].