Aflatoxins and Fluorescence in Brazil Nuts and Pistachio Nuts

Walter E. Steiner,* Katharina Brunschweiler, Esther Leimbacher, and Rosmarie Schneider

Kantonales Labor Zurich, P.O. Box 8030, Zurich, Switzerland

Brazil nut and pistachio nut samples from lots known to be contaminated with aflatoxins were visually inspected in detail. Kernels were split lengthwise in halves, and internally highly discolored kernels were analyzed individually. Highly contaminated Brazil nut kernels showed yellow fluorescence if illuminated with light at 360 nm. However, not all fluorescent kernels were contaminated with aflatoxins, and brown spotted kernels also contained these toxins. While contaminated Brazil nut kernels showed different appearance, only brown or brown spotted pistachio kernels were highly contaminated with aflatoxins. In yellowish fluorescent pistachio kernels no aflatoxins, citrinin, kojic acid, patulin, or ochratoxin A could be detected (detection limits: 10–100 μg/kg, 0.5–1, 40–50, 0.2–0.5, and 0.1–0.3 mg/kg, respectively). Slightly discolored kernels and sound kernels were analyzed in different batches. In this way it was shown that contaminated kernels were internally discolored. Ratios of 4700 uncontaminated kernels to 1 kernel containing aflatoxin B_1 and 4300 to 1, respectively, were calculated for two pistachio nut samples from different lots. A similar ratio for Brazil nuts could not be calculated since not all highly contaminated kernels were analyzed individually. The highest aflatoxin B_1 concentration in a pistachio kernel was 1400 mg/kg; in a Brazil nut 4 mg/kg aflatoxin B_1 and 1.2 mg/kg aflatoxin G_1 were detected. The high aflatoxin concentrations in a few kernels indicate that the usual sampling sizes of 20 and 50 lb (Brazil nuts and pistachio nuts, lots < 200 bags and 75 000 lb, respectively) may not be sufficient.

The uneven distribution of aflatoxins in pistachio nuts causes severe analytical problems. Even with 24-kg samples, Dickens and Welty (1975) found considerable variation among replicated aflatoxin test results from a contaminated lot. Apparently, few nuts contained high concentrations of aflatoxins. Sampling errors in testing pistachio nuts for aflatoxin contamination were similar to those found in shelled peanuts (Dickens and Welty, 1975). During the past 3 years, we analyzed several samples of pistachio nuts certificated as being free of aflatoxins; we found aflatoxin concentrations above 90 μg/kg. Aflatoxin contents in homogenized samples of Brazil nuts from the same lot did not vary in the same wide range as in homogenates of pistachio nuts but pointed also to single kernels being highly contaminated.

Shotwell et al. (1972, 1974), Fennell et al. (1973), and Steiner et al. (1991) found a relationship between BGY fluorescence and aflatoxin contamination in single corn kernels. Similar results were found in dried figs (Steiner et al., 1988; Reichert et al., 1988). To determine whether such a relationship existed in nut kernels, samples of pistachio and Brazil nuts from lots known to be contaminated with aflatoxins were analyzed in more detail.

MATERIALS AND METHODS

Selection of Kernels. Brazil nut kernels and pistachio nuts were cut lengthwise with a knife. A Blak Ray long-wave ultraviolet lamp (Model B-100; 360 nm) was used to detect fluorescence. UV-prospective spectacles and medical gloves were worn. The aim was to find all highly contaminated kernels in the samples. For this reason, internally highly discolored kernels were picked for analysis. According to the experience with these kernels, discolored kernels expected to contain no or low aflatoxin concentrations were collected to small batches for analysis. If aflatoxins were not detected or were present only in low concentrations, larger batches of kernels with similar appearance were collected and analyzed. Sound kernels were analyzed as large batches.

Brazil Nuts. The analysis of 8 kg of shelled Brazil nuts from Brazil (crop 1988) indicated a contaminated lot; 42.286 kg of this same lot was analyzed in several portions (Figure 1).

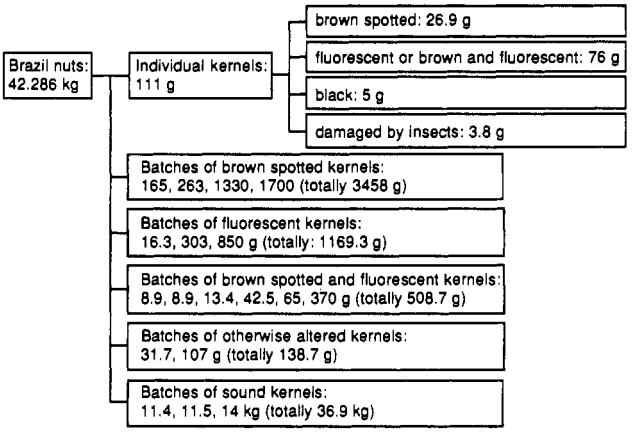

Figure 1. Brazil nuts separated into batches of brown spotted, fluorescent, brown and fluorescent, otherwise altered, and sound kernels. Otherwise altered kernels showed a greenish color, blue or white fluorescence.

Pistachio Nuts. Crop 1988: 4 kg (sample 1); 2.4 kg (sample 2); 9 kg (sample 3); 4.8 kg (sample 4). Crop 1989: 36.6 kg (14.8 kg of this quantity was analyzed in more detail as sample 5). Sample 1 originated from Turkey; samples 2–5 were from Iran. Sample 1–3 were divided into parts with and without fluorescent shells. In samples 3–5 kernels were classified as brown or brown spotted, yellow fluorescent, otherwise altered, or sound (Figure 2, sample 3 and 5).

Totals of 8.5 (13 individual fluorescent kernels) and 88.5 g (10 portions of 10 fluorescent kernels) were tested for citrinin, ochratoxin A, and patulin; 1.63 g (2) of fluorescent kernels and 5.35 g (12) of brown or brown spotted kernels tested for aflatoxins were also tested for kojic acid.

Samples of 10.8 and 11 kg, corresponding to the same lot as sample 5, were homogenized with shells.

The mean weight of the Brazil nuts analyzed was 4.56 g (n = 100), the mean weight of a pistachio kernel was 0.596 g (n = 98). The weight of a shell was 0.452 g (n = 98) and of the whole nut 1.045 g.

Standard Solutions. Aflatoxins, citrinin, and ochratoxin A were obtained from Serva GmbH and Co.; kojic acid was a product of Fluka Chemie AG.

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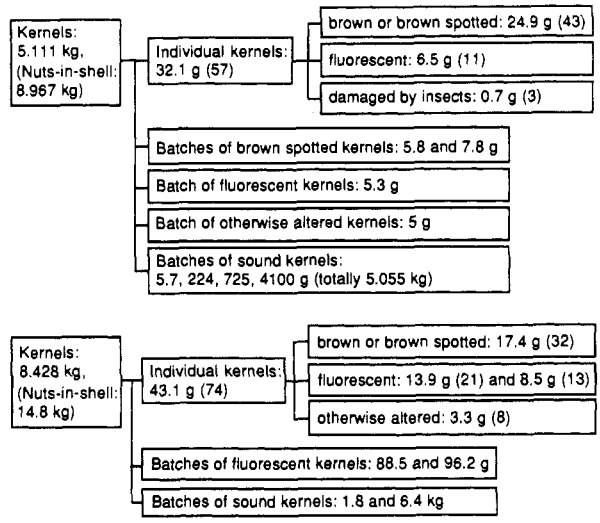

Figure 2. Pistachio kernels (upper diagram, sample 3; bottom, sample 5) separated into batches of brown or brown spotted, fluorescent, otherwise altered, and sound kernels. Otherwise altered kernels showed blue fluorescence, very small size, black spots, or damage by insects.

Aflatoxins B_1, B_2, G_1, and G_1. A solution in toluene/acetonitrile (98:2), containing 0.5 μg/mL aflatoxins B_1 and G1 as well as 0.25 μg/mL aflatoxins B_2 and G2 was prepared and assayed according to AOAC Method 26.009 (Stoloff and Scott, 1984). Ochratoxin A and patulin were assayed according to AOAC Methods 26.113 and 26.128 (Stoloff and Scott, 1984). Stock solutions of 200 μg/mL citrinin and patulin in ethanol and 40 μg/mL ochratoxin A in benzene/acetic acid were diluted to 1, 2, and 1 μg/mL, respectively, before analysis.

Kojic acid was present at 2 μg/mL in water/methanol (9:1).

Equipment. The fluorodensitometer was a Camag-TLC-scanner Mk. II. HPLC pumps Merck Hitachi Model 665A-12 8, Kontron T 414, were used. The UV detector was a Kratos Spectroflow 773 (UV) and fluorescence detector a Merck Hitachi F 1050. A 10-port switching valve C10W, with electric actuator (Valco), was used. The cutter was a Stephan UM12, operated at 1500 rpm (Stephan u. Söhne GmbH and Co., Hameln, Germany).

Color slides were made with Fujichrome 400 ASA using a Minolta XE-1 camera (lens 1:3.5, f = 100 mm, aperture 11, exposure time 3 min). A Cokin-A 173 filter was used in series with a polarization filter. Polarization was adjusted to the position transparent for orange/red light. The kernels were illuminated with a Blak Ray lamp (see above).

Homogenization of Nuts. Batches of nuts were homogenized in a cutter (some of the pistachio nuts were crushed with shells). To achieve an even distribution of aflatoxins, the process was only stopped after about 10 min when the sample turned to an oily homogenate.

Extraction. *Homogenized Batches of Nuts.* Eighty grams of homogenate was placed in a Waring Blendor. Methanol (200 mL) and water (20 mL) were added to the Blendor, and the mixture was homogenized for 3 min. After 60 mL of water was added, the slurry was again homogenized for 3 min. The resulting mixture was filtered through fluted filter paper; 70 mL of filtrate was collected and transferred into a separatory funnel. Hexane (50 mL), water (55 mL), and sodium chloride (2 g) were added, and the mixture was shaken for 1 min. After removal of the organic layer, 90 mL of dichloromethane was added to the separatory funnel, the mixture shaken, and the organic layer drained off and dried with anhydrous sodium sulfate. The solvent was evaporated on a rotary evaporator (ca. 20 Torr, 40 °C). The residue was redissolved in 1 mL of toluene/acetonitrile (98:2) and used for thin-layer chromatographic analysis.

Single Kernels. Extraction of Aflatoxins. The following procedure was used for Brazil nuts: Single kernels were ground with a pestle in a mortar. Aflatoxins were extracted twice with 35 mL of methanol water (7:3). The extracts were filtered as the homogenates described above. To 35 mL of filtrate were added 25 mL of hexane, 30 mL of water, and 1 g of sodium chloride. The mixture was shaken for 1 min. The organic layer was withdrawn; 45 mL of dichloromethane and 10 mL of water were added to the aqueous layer. The mixture was shaken for 2 min. The organic layer was dried with anhydrous sodium sulfate and treated as above. The same method was applied for pistachio kernels; however, extraction involved half the quantities of reagents.

Extraction of Citrinin, Patulin, and Ochratoxin A from Individual Pistachio Nuts. In a mortar, kernels were extracted twice with 20 mL of acetonitrile (each time 3 min). The extracts were filtered and defatted with 20 mL of hexane, and the acetonitrile layer was evaporated at 40 °C and 20 Torr. The residue was redissolved in 1 mL of mobile phase.

Extraction of Kojic Acid. Kojic acid was extracted together with aflatoxins. The solvent was methanol/water (7:3).

Chromatography and Quantitation. *Aflatoxins.* The extracts to be assayed and the respective standard solutions were spotted on a line ca. 12 cm from the bottom of commercial silica sheets (Merck No. 5553), activated immediately before use for 30 min at 100 °C, and the first development was carried out in anhydrous diethyl ether over the whole distance. The portion of the plate from ca. 13.5 cm upward, containing substances separated from aflatoxins, was cut off, and the plate was redeveloped in the opposite direction with a mixture of chloroform/acetone/water (88:12:0.2). The spots on the plates were quantitated with a fluorodensitometer: an excitation wavelength of 365 nm and a 400-nm cutoff filter on the emission side were used. The results are not corrected for recoveries, which were between 60 and 90% at a spiking level of 1.25 μg/kg. For batches of nuts, the detection limits were 0.5 ppb for each toxin, for Brazil nut kernels 0.5–2 μg/kg, and for pistachio nut kernels 10–100 μg/kg, respectively.

Citrinin and Ochratoxin A. A deltabond octyl column (4.6 mm i.d. × 25 cm; 5-μm particle size; Keystone Scientific, Inc., Bellefonte, PA) was used. The mobile phase consisted of 2 M phosphoric acid/methanol (35:65). The separation was performed at ambient temperature at a flow rate of 1 mL/min. All injections (20 μL) were made by using a filled-loop technique. The fluorescence detector was operated at an excitation wavelength of 360 nm and an emission wavelength of 500 nm. Chromatographic peaks were quantitated relative to the area of external standards. Detection limits: 0.5–1 and 0.1–0.3 mg/kg, respectively. Recovery: 25 and 50%, respectively, at a spiking level of 2 mg/kg.

Patulin. A Lichrospher RP 18 column (4 mm i.d. × 12.5 cm, 5-μm particle size, Merck) with precolumn (4 mm i.d. × 2 cm) was used with water/acetonitrile (96:4) as mobile phase at 1.3 mL/min (ambient temperature). The injection volume was 20 μL. Patulin was detected at 276 nm and quantitated using external standards. Detection limit: 0.2–0.5 mg/kg. Recovery: 85% at a spiking level of 2 mg/kg.

Kojic Acid. Using nitrogen, 50 μL of extraction solution (standard solution) was blown to dryness; 50 μL of acetic anhydride and 50 μL of pyridine were added to the residue, and kojic acid was acetylated for 30 min at ambient temperature. The reaction solution was blown to dryness as above and redissolved in 1 mL of mobile phase, consisting of water/acetonitrile (9:1). A Hypersil ODS column (4 mm i.d. × 12.5 cm, 5-μm particle size, Shandon) was used. The injection valve was equipped with a 20-μL sample loop. The flow rate was 1 mL/min, and separation was performed at ambient temperature. Detection wavelength was 250 nm. The derivatized standard solution was used as external standard. Detection limit: 40–50 mg/kg.

RESULTS AND DISCUSSION

Distribution of Aflatoxins in a Lot of Brazil Nuts. In halved Brazil nut kernels, mainly brown areas and, in fewer cases, yellow fluorescence were observed at the internal parts (Figure 3). The spots with yellow fluorescence were only visible if kernels were illuminated with light at 360 nm. In normal light, these fluorescent areas appeared as brown spots. Other anomalies such as white fluorescence, black spots, and damage by insects were seldom observed.

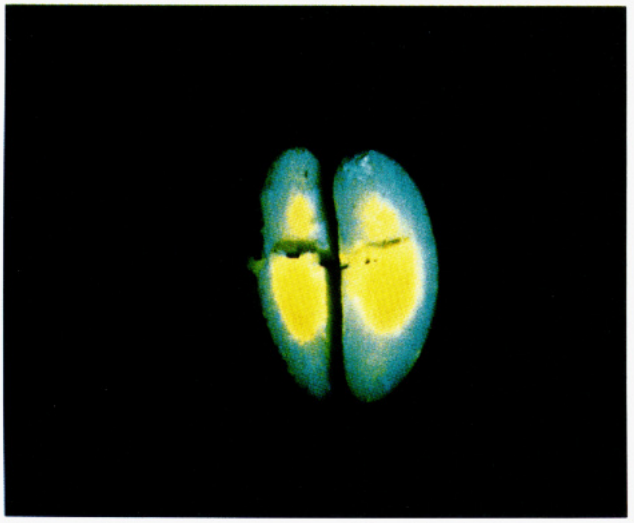

Figure 3. Picture of a contaminated Brazil nut illuminated with light at 360-nm wavelength. In daylight, the yellow fluorescent spots appeared brown. However, not all spots perceived as brown in normal light were fluorescent at 360-nm wavelength. The kernel shown contained 930 μg/kg aflatoxin B_1 and 760 μg/kg aflatoxin G_1.

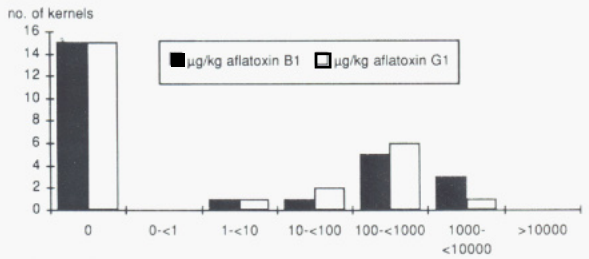

Figure 4. Distribution of aflatoxins in 25 individually analyzed Brazil nuts showing yellow fluorescence (UV light), dark brown spots (daylight), black color, or damage by insects.

Table I. Individually Analyzed Brazil Nut Kernels Containing Aflatoxins

	aflatoxin, (μg/kg)/μg			
kernel wt, g	B_1	B_2	G_1	G_2
4	3/0.01		3/0.01	
4.2	60/0.25	10/0.04	20/0.08	
5.2	180/0.94	20/0.10	230/1.20	50/0.26
4.2	190/0.80	40/0.17	70/0.29	10/0.04
4.5	370/1.67	30/0.14	560/2.52	10/0.05
5.2	430/2.24	90/0.47	600/3.12	70/0.36
4.4	930/4.09	140/0.62	760/3.34	60/0.26
0.7	1000/0.70	190/0.13	290/0.20	30/0.02
4.2	1090/4.58	240/1.01	900/3.78	130/0.55
5.5	4180/23.0	640/3.52	1200/6.60	50/0.28
total aflatoxin, μg	38	6	21	2

Seventeen fluorescent kernels, six kernels with strong brown color, a black kernel, and a kernel damaged by insects were analyzed individually. About 60% of these kernels were free of aflatoxins; in the remaining 40%, aflatoxin B_1 was found in concentrations between 3 and 4200 μg/kg (Figure 4; Table I). All individually analyzed kernels containing aflatoxins showed yellow fluorescence at the cut surface. Of the 17 fluorescent kernels, only 10 (59%) were contaminated with aflatoxins. The aflatoxin concentrations in the kernels are shown in Table I. All individually analyzed kernels containing aflatoxin B_1 also contained aflatoxin G_1.

The six dark brown spotted kernels were not contaminated with aflatoxins. Therefore, we assumed the brown spots in kernels to be no criterion to recognize contaminated kernels. A further analyzed batch of 263 g of brown spotted kernels contained 1 μg/kg aflatoxin B_1 and 1.9 μg/kg aflatoxin G_1 and seemed to confirm that these kernels were not heavily contaminated. However, further batches of 165, 1330, and 1700 g showed the following concentrations: 33, 9.4, and 1.6 μg/kg aflatoxin B_1 and 31, 19, and 1.8 μg/kg aflatoxin G_1, respectively. Possibly, not all fluorescent kernels had been eliminated, maybe since the fluorescence did not reach the cut surface of the kernels. However, highly contaminated kernels without fluorescence or a more frequent but lower toxin concentration in brown kernels could also explain the phenomenon.

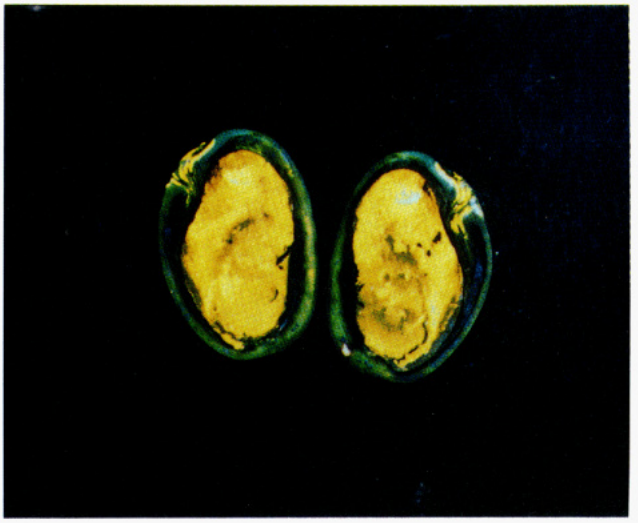

Figure 5. Picture of the pistachio kernel with the highest aflatoxin concentration found (1400 mg/kg aflatoxin B_1) illuminated with light at 360 nm. A typical yellow fluorescence as observed with some pistachio kernels or Brazil nuts was absent. The brown color was seen at daylight as well as at 360 nm.

Eight further batches with weights ranging from 8.9 to 370 g contained aflatoxin concentrations ranging from 2 to 337 μg/kg (the latter result originating from two kernels). Sound kernels (totally 36.9 kg) were free of aflatoxins.

The aflatoxin concentrations as a weighted mean of the total sample, the brown, and the fluorescent kernels are shown in Table II. Sixty-nine percent of the aflatoxin B_1 present in the whole sample of 42.286 kg was in fluorescent kernels (1754 g) and 31% in brown spotted kernels (3484 g). Correspondingly, 48% of the total aflatoxin G_1 was in fluorescent kernels and 52% in brown spotted kernels. Just 1 of 12 analyzed batches of Brazil nut kernels containing aflatoxins was contaminated exclusively with aflatoxins B_1 and B_2, which indicates that kernels were mainly infected by *Aspergillus parasiticus*.

Distribution of Aflatoxins in Several Lots of Pistachio Nuts. In UV light at 360 nm, two samples of pistachio nuts were split into nuts with and without fluorescent shells. All four fractions were analyzed for aflatoxins. In sample 1, 8% of the nuts showed fluorescence but were free of aflatoxins, while the 9% of fluorescent nuts in sample 2 contained 47 μg/kg aflatoxin B_1 (Table III). The nonfluorescent portion of sample 1 was free of aflatoxins, while that of sample 2 contained 80% of the total aflatoxin content. These results are in agreement with those of Dickens and Welty (1975), who split 46 samples into fluorescent and nonfluorescent nuts and analyzed these portions separately. In 8% of the fluorescent portions they found no aflatoxins. In 54% of the samples, the aflatoxin concentrations in the nonfluorescent portion exceeded that in the fluorescent one. These results suggested that the analysis of nuts with fluorescent shells was not an appropriate means to find all kernels containing high aflatoxin concentrations.

Table II. Concentrations of Aflatoxins in Separated Brazil Nut Kernels According to Visual Appearance[a]

	total sample	brown kernels	yellow fluorescent kernels	otherwise altered kernels	sound kernels
kernels, g/%	42286/100	3484/8.2	1754/4.1	148/0.4	36900/87.3
aflatoxin B_1, (μg/kg)/μg	1.57/66.5	6/20.9	26/45.6	nd[b]	nd
aflatoxin B_2, (μg/kg)/μg	0.31/13.3	0.8/2.8	6/10.5	nd	nd
aflatoxin G_1, (μg/kg)/μg	1.57/66.5	10/34.9	18/31.6	nd	nd
aflatoxin G_2, (μg/kg)/μg	0.37/15.8	3/10.5	3/5.3	nd	nd

[a] Since resulting groups of kernels were analyzed in several batches, the aflatoxin concentrations indicated are weighted means. [b] Not detected. Detection limit: 0.5–2 μg/kg, according to analyzed quantity.

Table III. Comparison of Aflatoxin Concentrations in Three Samples from Different Lots of Pistachio Nuts with Fluorescent Shells

	pistachio nuts in shell, g/%			aflatoxin B_1, (μg/kg)/μg		
sample no.	total	part with fluorescent shells	part without fluorescent shells	whole sample	part with fluorescent shells	part without fluorescent shells
1	4000/100	315/8	3685/92	<0.5	<0.5	<0.5
2	2444/100	225/9	2219/91	21.6/52.8	47/10.6	19/42.2
3	8967/100	1271/14	7696/86	2.6/23		

Table IV. Aflatoxin Concentrations (>100 μg/kg) in Individual Pistachio Kernels and Corresponding Shells

sample no.	kernel wt, mg	concn of aflatoxin B_1, (μg/kg)/μg, in		concn of aflatoxin B_2, μg/kg, in	
		kernel	shell	kernel	shell
3	680	18000/12		2000	
	670	16500/11		2500	
5	590	1400000/826	1400/0.8	145000	260
	320	218000/70	32/0.01	23000	nd
	730	13000/9	nd[a]	1300	nd

[a] Not detected. Detection limit: ca. 10 μg/kg.

According to our experience with Brazil nuts, it seemed possible that the main part of the aflatoxin was located in brown or fluorescent kernels. Since the surface of the intact kernels did not fluoresce, the nuts were halved. Kernels were classified as the Brazil nuts: kernels with brown spots or brown color throughout the nut (Figure 5), those with yellow fluorescence upon illumination at 360 nm, and kernels with some extra symptoms such as blue fluorescence and damage by insects. Kernels with brown and fluorescent spots were classified as fluorescent. Many investigated nuts had a fluorescent area on the inside of the shells at the point the shells were attached to the tree (Figure 5). Possibly this tissue is a favorable area for colorization by mold. The situation might be analogous to that in a tip cap (pedicel) of corn kernels, which is known to be easily infected by fungi (Marsh et al., 1984).

From sample 3, which consisted of 5111 g of pistachio kernels, 57 internally discolored kernels were sorted out for individual analysis; 43 of these kernels were brown or brown spotted, 11 exhibited yellow fluorescence, and 3 were damaged by insects. Aflatoxins were only present in the brown or brown spotted kernels; however, only two of these kernels were highly contaminated (Table IV). The homogenized batches of fluorescent (5.3 g), otherwise altered (5 g), and sound kernels (5055 g) contained no aflatoxins. Sample 5 showed similar results: from 8428 g of kernels, 32 brown or brown spotted, 21 fluorescent, and 8 otherwise altered kernels were picked out and analyzed individually for aflatoxins. Only three brown kernels were highly contaminated (Table IV). The remaining parts of the sample (184.7 g of fluorescent and 8.2 kg of sound kernels) were free of aflatoxins. All of the aflatoxin-containing pistachio kernels seemed to be infected by *Aspergillus flavus* since no aflatoxins G_1 and G_2 could be detected.

Dickens and Welty (1975) could not detect aflatoxins in separately analyzed shells from contaminated samples. The shells of the contaminated single kernels analyzed in our laboratory contained low aflatoxin concentrations compared to the corresponding kernels (Table IV). It may be concluded that shells do not seem to be a convenient substrate for fungi to produce aflatoxins. Therefore, the analysis of a sample does not necessarily need separation of the shells before homogenization since shells do not contribute to a considerable concentration of aflatoxins in the sample.

Other Mycotoxins in Pistachio Nuts. No aflatoxins were detected in pistachio kernels exhibiting yellow fluorescence at internal parts. As hyphae were observed in some kernels, other toxin-producing fungi seemed possible. The following reasons prompted us to determine the mycotoxins citrinin, ochratoxin A, and patulin: citrinin is a yellow fluorescent substance, ochratoxin A was found in dried figs exhibiting BGY fluorescence, and patulin was detected in yellow fluorescent almonds. However, none of the toxins were detected in 113 fluorescent kernels. As the mean weight of a pistachio kernel was only 0.596 g, the detection limits were 0.5–1, 0.1–0.3, and 0.2–0.5 mg/kg, respectively. The aim was to find toxin concentrations at levels similar to those of aflatoxins.

Most strains of *A. flavus* produce kojic acid together with aflatoxins (Parrish et al., 1966), and kojic acid is supposed to be a precursor of the BGY fluorescent substance (Marsh et al., 1969; Fennell et al., 1973; Shotwell et al., 1974). Twelve brown colored and two fluorescent kernels were analyzed for this toxin, but even in the nuts contaminated with aflatoxins, kojic acid was not detected (detection limit: 40–50 mg/kg). Since concentrations of 800 mg/kg and higher were found in maize (Oeschlmüller, 1987; Steiner 1991), we expected the toxin to be in a similar concentration range. It is possible that conditions to produce kojic acid are not optimal in pistachio nuts. In a synthetic substrate with 0.04% nitrogen it was shown that besides aflatoxins no kojic acid was produced by strains of *A. flavus* (Schröppel and Müller, 1978).

Reduction of Aflatoxin Content by Sorting Out Critical Kernels. The Brazil nut sample analyzed in detail showed aflatoxins in brown spotted or fluorescent kernels. After these kernels were removed, the rest of the sample (87.7%, Table II) was free of aflatoxins.

In pistachio nuts, the aflatoxins were located in brown or brown spotted kernels; 99.2% of sample 3 and 99.8% of sample 5 remained without aflatoxins after elimination of this fraction. This demonstrates the brown color as a criterion to find pistachio kernels containing high aflatoxin

Table V. Concentrations of Aflatoxins in Separated Shelled Pistachio Kernels According to Visual Appearance[a]

	kernels, g/%				aflatoxin B_1, μg/kg, in				
sample no.	total	brown or brown spotted	yellow fluorescent	otherwise altered	total sample	brown kernels	yellow fluorescent kernels	otherwise altered kernels	sound kernels
4	2719/100	2.5/0.1	46.8/1.7		<0.5	<0.5	<0.5		<0.5
3	5111/100	38.5/0.8	11.8/0.2	5.7/0.1	5	600	nd[b]	nd	<0.5
5	8428/100	17.4/0.2	207/2.5[c]	3.3/0.04	107	52000	nd	nd	<0.5

[a] The three samples originated from different lots. Since resulting groups of kernels were analyzed in several batches, the aflatoxin concentrations indicated are weighted means. [b] Not detected. Detection limit of single kernels: 10–100 μg/kg. [c] The presence of aflatoxins was controlled in 110 g, the remaining part was analyzed for citrinin, ochratoxin A and patulin.

concentrations. However, it was not possible to recognize which of these kernels were contaminated with aflatoxins.

One of 10 contaminated and individually analyzed Brazil nuts and 1 of 5 pistachio nuts, respectively, showed a remarkably lower weight than the mean weights determined in the samples (Tables I and IV; mean kernel weights of 4.54 and 0.596 g, respectively). Therefore, 13 of 15 contaminated kernels did not seem to be damaged to such an extent to show an essentially lower weight than average. The main contamination in these samples would not have been reduced by eliminating small kernels.

Farsaie et al. (1981) developed an automatic sorter for removing pistachio nuts with fluorescent shells. This is a means to reduce the aflatoxin content of a lot by ca. 50%. Contaminated nuts without fluorescent shells escape this control. The pistachio nut shell of the kernel containing 1400 mg/kg aflatoxin B_1 was not fluorescent.

Sampling. From a lot of pistachio nuts, totally 36.6 kg, including sample 5, was analyzed. A sample of 10.8 kg was contaminated with 22 μg/kg, a further sample of 11 kg was contaminated with 8 μg/kg, and sample 5 was contaminated with 61 μg/kg aflatoxin B_1 (14.8 kg, nuts in shell; total aflatoxin content was 905 μg, calculated from data of Table IV). The latter sample without the kernel containing 1400 mg/kg aflatoxin B_1 (Table IV) would have shown a concentration of 5.3 μg/kg aflatoxin B_1.

A ratio of 4300 uncontaminated kernels to 1 kernel containing aflatoxin B_1 can be calculated from sample 3 (2 kernels in 5.1 kg, Tables IV and V; mean weight of a kernel was 0.596 g). In sample 5, this ratio was 4700 to 1 (3 kernels in 8.4 kg). If nuts in shell are taken into account, samples 3 and 5 had one contaminated nut in 4.5 and 4.9 kg, respectively (mean nut weight: 1.045 g). Considering the differences in aflatoxin concentration on single kernels in these samples, the recommended sample size of the FDA (Campbell et al., 1986) seems to be insufficient for pistachio nuts (22.7 kg of nuts in shell; lot size of 34 tons). On the other hand, all samples containing aflatoxins were below 15 kg, which may indicate that a heavily contaminated lot can be recognized by a sample size of about 10 kg.

The Brazil nuts showed a more frequent but less extreme contamination with aflatoxin B_1 (Tables I and II). The 42.286 kg of kernels analyzed in more detail gave a weighted mean of 1.6 μg/kg aflatoxin B_1 and G_1, respectively. Another laboratory detected 8.4 μg/kg aflatoxin B_1 in a sample of the same lot. A further sample of 8 kg analyzed in our laboratory contained 4.2 μg/kg aflatoxin B_1 and 2.8 μg/kg aflatoxin G_1. The higher contents of aflatoxins in the batchwise-analyzed samples may have originated from higher contaminated kernels.

In the 42.3 kg of Brazil nuts under investigation, not all highly contaminated kernels were analyzed individually. According to the variation of aflatoxin concentrations in the homogenates of brown spotted kernels, few highly contaminated kernels as in the fluorescent fraction are probable. As a Brazil nut kernel has about the same weight as eight pistachio kernels, a sampling size of 9.1 kg of nuts in shell (lot size <200 bags) might be a minimum.

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