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Incidence, Level, and Behavior of Aflatoxins during Coffee Bean Roasting and Decaffeination

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Screening for aflatoxins (Afs), isolation and identification of *Aspergillus flavus*, and the effect of decaffeination and roasting on the level of contamination in coffee beans are studied. The percent frequency of *A. flavus* ranged between 4 and 80% in green coffee beans (GCB), whereas in ground roasted coffee beans (GRCB), it ranged between 1 and 71%. Aflatoxins were detected in 76.5 and 54.6% of the infected samples with averages of 4.28 and 2.85 μg/kg of GCB and GRCB, respectively. Roasting was demonstrated to lower the concentration of Afs in GCB. The Afs levels were reduced by ~42.2−55.9% depending on the type and temperature of roasting. The highest yields of Afs were detected in the decaffeinated green coffee beans (24.29 μg/kg) and roasted coffee beans (16.00 μg/kg). The growth of *A. flavus* in liquid medium containing 1 or 2% caffeine was reduced by 50%, and the level of aflatoxin in the medium was undetectable.

KEYWORDS: Aflatoxins; coffee; processing; roasting

INTRODUCTION

Many fungi produce poisonous substances called mycotoxins that can cause acute or chronic intoxication and damage (*1*). Mycotoxins are secondary metabolites produced by fungi colonizing cereal grains in the field and in storage (*1*−*3*). They are harmful to both humans and animals and remain an area of major concern throughout the world. Mycotoxins may reach consumers directly through consumption of a primary crop such as nuts, vegetables, and grains or indirectly through consumption of animal products from livestock exposed to contaminated feed.

The first group of mycotoxins that was isolated and described in 1961 was the aflatoxins (Afs), as the results of several acute animal disease outbreaks in 1960 (*4*). Aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* are the mycotoxins most extensively studied as potent animal carcinogens and therefore are suspected as a cause of cancer in man (*5*, *6*).

Coffee is one of the most widely consumed beverages in the world because of its pleasant taste, its pharmacological effects, and its stimulatory effects on mental and physical activity. The presence of aflatoxins in coffee is of great concern because they are potent naturally occurring hepatocarcinogens (*6*). Nakajima et al. and Blanc et al. (*7*, *8*) have reported the natural occurrence of mycotoxins in green coffee beans. The effect of heat treatment on the destruction of aflatoxins in green coffee beans is of great interest to the coffee industry. Several studies have indicated that aflatoxins in contaminated coffee beans have to be degraded by heat treatment (*9*, *10*).

Microwave roasting can be used as an alternative method to destroy aflatoxins. Staron et al. (*11*) treated contaminated peanuts for 4 min in a microwave oven, resulting in 95% reduction of aflatoxins. Niola et al. (*12*) used the microwave to destabilize patulin. Farag et al. (*13*) reported that microwaves were used to destroy aflatoxins in yellow corn and groundnuts contaminated with aflatoxins.

The previously mentioned studies prompted us to provide information on the aflatoxin level in green coffee beans and ground roasted coffee and to evaluate and identify molds. In addition, our studies were conducted to determine the effect of roasting on aflatoxin reduction, as well as the effect of caffeine and the decaffeination procedure on the growth of aflatoxin-producing molds as well as aflatoxin production.

MATERIALS AND METHODS

Samples. Thirty samples of green coffee beans (GCB) and ground roasted coffee beans (GRCB) were purchased from local markets at Giza and Cairo.

Mycological Studies. *Green Coffee Beans.* One hundred beans of each sample were surface disinfected with 5% sodium hypochlorite (NaOCl) for 1 min and rinsed three times with sterile water. The beans were plated on malt extract agar medium having the following composition per liter of distilled water: 20 g of powdered malt extract, 1 g of glucose, and 20 g of agar.

Ground Roasted Coffee Beans. Serial dilutions of samples were made and immediately dispensed into a sterile Petri dish containing malt extract agar. The plate was incubated for 5−7 days at 28 °C.

Identification of different isolates to the genera or species was conducted according to the methods of Gilman et al., Barnett et al., and Nelson et al. (*14*−*16*). Attention was focused on *A. flavus*, and the percent of occurrence was determined.

Roasting Procedures. An experiment was conducted with green coffee beans, which had been previously identified as containing a high amount of Afs (8.92 μg/kg total Afs). Roasting was carried out using three methods: (A) traditional roasting at 180 °C for 10 min; (B) oven-roasting for 15 min at 150 °C; and (C) household microwave oven roasting (Sharp carousel microwave oven, model R-3A58) for 4 min.

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Table 1. Occurrence of *A. flavus* and Aflatoxin Production in GCB[a] Compared with YES[b] Medium

	occurrence %		μg/kg of GCB					μg/L of YES				
sample	*A. flavus*	others	B_1	B_2	G_1	G_2	total	B_1	B_2	G_1	G_2	total
1	72	28	6.00	0.57	2.10	0.25	8.92	8.29	0.69	2.75	0.17	11.90
2	45	55	1.52	0.35	0.59	ND[c]	2.46	4.75	ND	3.45	ND	8.20
3	0	100	ND	ND	ND	ND	ND					
4	40	60	ND	ND	ND	ND	ND	0.31	ND	0.79	ND	1.10
5	23	77	2.86	0.96	ND	ND	3.82	6.19	ND	3.26	ND	9.45
6	0	100	ND	ND	ND	ND	ND					
7	0	100	ND	ND	ND	ND	ND					
8	45	55	ND	ND	ND	ND	ND	1.73	0.65	2.92	ND	5.30
9	0	100	ND	ND	ND	ND	ND					
10	30	70	4.29	1.56	ND	0.39	6.24	6.99	2.15	2.15	3.28	14.57
11	80	20	4.77	0.65	2.72	0.13	8.27	3.87	2.60	ND	1.18	7.65
12	0	100	ND	ND	ND	ND	ND					
13	4	96	2.82	0.25	0.96	ND	4.03	8.58	0.36	4.64	ND	13.58
14	8	92	2.43	0.95	ND	ND	3.38	2.00	0.70	1.60	0.50	4.80
15	13	87	0.83	ND	ND	ND	0.83	18.90	0.75	0.99	4.50	25.14
16	42	58	3.60	1.14	1.67	ND	6.41	3.04	1.98	11.78	ND	16.80
17	0	100	ND	ND	ND	ND	ND					
18	0	100	ND	ND	ND	ND	ND					
19	7	93	1.42	0.13	0.52	ND	2.07	3.72	1.44	0.70	1.75	7.61
20	0	100	ND	ND	ND	ND	ND					
21	0	100	ND	ND	ND	ND	ND					
22	0	100	ND	ND	ND	ND	ND					
23	0	100	ND	ND	ND	ND	ND					
24	8	92	ND	ND	ND	ND	ND	0.80	ND	ND	ND	0.80
25	0	100	ND	ND	ND	ND	ND					
26	73	27	2.90	1.23	1.67	ND	5.80	1.53	0.71	1.68	0.87	4.79
27	10	90	0.57	ND	0.19	ND	0.76	0.98	ND	0.67	ND	1.65
28	5	95	ND	ND	ND	ND	ND	3.75	ND	ND	ND	3.75
29	0	100	ND	ND	ND	ND	ND					
30	33	67	1.78	0.16	0.65	ND	2.59	1.70	0.97	1.15	1.79	5.61

[a] GCB, green coffee beans. [b] YES, yeast extract sucrose. [c] ND, not detectable.

Decaffeination Procedure. Both green and roasted coffee beans were decaffeinated according to the method of Nartowicz et al. (*17*). Each sample (1 kg) was decaffeinated by boiling the beans in 1.0 L of water for 5 h, changing the water hourly. The decaffeinated beans were dried in an oven at 120 °C for 3−4 h.

Caffeine Analysis. Caffeine levels were determined using the official, first-action method of coffee (*18*) and reported as milligrams of caffeine per gram of coffee (dry weight).

Toxin Production. *Liquid Media.* The ability of *A. flavus* strains (positive or negative) that were isolated from collected samples using yeast extract sucrose (YES) medium to produce Afs was investigated. The isolated strains were regenerated on potato dextrose agar (PDA) slants at 28 °C for 1 week to obtain sufficient inoculums. The medium (100 mL of YES) was inoculated with 1 mL (10^6) of spore suspension and then incubated at 28 °C for 2 weeks (*19*).

Coffee Beans. Flask cultures were prepared by transferring 50 g of whole coffee beans (natural and decaffeinated of both green and whole roasted coffee beans) to 250 mL Erlenmeyer flasks, which were then inoculated with 1.0 mL of spore suspension (10^6) of *A. flavus*, NRRL 3357 (Northern Regional Research Laboratory, USDA). The flasks were incubated for 15 days at 28 °C.

Aflatoxin Analysis. *Samples.* Afs were extracted from coffee (natural, decaffeinated of both green and roasted coffee beans, and ground roasted coffee) according to the method of Kamimura et al. (*20*). The determination of Afs was performed using high-performance liquid chromatography (HPLC) (*21*). HPLC analysis was performed using a Waters liquid chromatography system model 6000A, system controller (model 720), data module (model 730), U6K injector, and fluorescence detector model 420. The detection wavelengths were excitation 338 nm and emission 455 nm. Separation was on a C_{18} reverse phase column (5 μm, 250 mm × i.d. 4.6 mm). The mobile phase consisted of solvent A (acetonitrile + water, 23 + 77, v/v) and solvent B (methanol).

Liquid Medium (YES). At the end of the inoculation period, fungal mycelia were removed by filtration. The filtrate were brought to pH 2 with HCl and extracted with chloroform ($CHCl_3$, 3 × 100 mL). The combined extracts were dried using anhydrous sodium sulfate and evaporated to dryness. Afs were determined by HPLC.

Effect of Caffeine on the Growth of Aflatoxin-Producing Molds and Afs Production. *A. flavus* strain (NRRL 3357) was grown in Erlenmeyer flasks containing 50 mL of YES medium supplemented with caffeine at concentrations of 0.0, 0.1, 0.5, 1.0, and 2.0% (w/v). They were incubated for 3, 6, 9, 12, 15, 18, and 21 days at 28 °C. After incubation, the mycelia mat was separated from YES culture by filtration using filter paper (no. 4). The mycelia were washed twice with ~100 mL of distilled water and dried overnight at 100 °C to constant weight to measure the fungal growth. The filtrates were analyzed for Afs and determined using HPLC as mentioned above.

RESULTS AND DISCUSSION

Fungi accompanying GCB and GRCB were isolated from all samples, and the amount of Afs in each sample was determined. Data are presented in **Tables 1** and ***2***.

Data in **Table 1** show that *A. flavus* was isolated from 17 of 30 samples with more than 56% of GCB contaminated with *A. flavus*. The frequency of its isolation ranged from 4 to 80%. Determination of aflatoxins in the infected samples (17 samples) with *A. flavus* revealed that concentrations ranged between 0.76 and 8.92 μg/kg. Moreover, all strains of *A. flavus* isolated from GCB were able to produce aflatoxins in liquid media. The total amount of Afs produced ranged between 0.8 and 25.14 μg/L of media (YES).

Concerning the ground roasted coffee beans (**Table 2**), *A. flavus* was isolated from 22 (73.3%) of 30 samples with frequency ranging between 1 and 71%. In particular, 12 of 22 infected samples were positive for Afs at levels ranging between 0.79 and 5.08 μg/kg of GRCB. About 86.4% of *A. flavus* isolates (19 of 22) produced Afs to some degree in liquid medium (YES); the amount of total Afs varied from 1.9 to 35.23 μg/L

Table 2. Occurrence of *A. flavus* and Aflatoxin Production in GRCB[a] Compared with YES[b] Medium

	occurrence %		μg/kg of GRCB					μg/L of YES				
sample	*A. flavus*	others	B_1	B_2	G_1	G_2	total	B_1	B_2	G_1	G_2	total
1	63	37	2.70	0.67	1.37	0.32	5.06	11.70	2.16	6.63	0.34	20.83
2	0	100	ND	ND	ND	ND	ND					
3	3	97	ND	ND	ND	ND	ND	0.79	0.46	ND	1.78	3.03
4	3	97	ND	ND	ND	ND	ND	2.11	ND	3.97	ND	6.08
5	8	92	ND	ND	ND	0.79	0.79	4.90	1.20	1.40	2.57	10.07
6	4	96	1.33	0.67	ND	ND	2.00	ND	0.61	3.20	1.93	5.74
7	63	37	2.12	0.87	ND	ND	2.99	5.80	3.20	ND	ND	9.00
8	0	100	ND	ND	ND	ND	ND					
9	6	94	ND	ND	ND	ND	ND	10.00	7.60	ND	2.50	20.10
10	2	98	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	9	91	ND	ND	ND	ND	ND	ND	1.32	ND	3.80	5.12
12	59	41	0.89	0.70	ND	ND	1.59	2.78	1.67	ND	1.58	6.03
13	4	96	ND	ND	ND	ND	ND	ND	2.78	3.97	14.40	21.15
14	71	29	1.17	ND	0.80	ND	1.97	5.80	3.12	ND	14.88	23.80
15	0	100	ND	ND	ND	ND	ND					
16	0	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
17	1	99	ND	ND	ND	ND	ND	ND	1.90	ND	ND	1.90
18	0	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
19	22	78	ND	ND	1.30	0.60	1.90	0.52	ND	5.37	11.61	17.50
20	23	77	0.37	ND	ND	1.72	2.09	ND	3.70	ND	2.90	6.60
21	3	97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
22	3	97	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
23	5	95	ND	ND	ND	ND	ND	ND	ND	5.21	2.92	8.13
24	0	100	ND	ND	ND	ND	ND					
25	52	48	0.73	0.52	ND	2.17	3.42	ND	ND	4.23	6.81	11.04
26	0	100	ND	ND	ND	ND	ND					
27	0	100	ND	ND	ND	ND	ND					
28	52	48	3.01	0.67	1.27	0.13	5.08	6.77	0.48	1.93	11.01	20.19
29	45	55	0.36	1.52	2.91	ND	4.79	2.92	7.39	19.60	5.32	35.23
30	32	68	ND	ND	ND	2.68	2.68	ND	ND	ND	3.70	3.70

[a] GRCB, ground roasted coffee beans. [b] YES, yeast extract sucrose. [c] ND, not detectable.

of media (YES). Only 4 (18.2%) of 22 *A. flavus* isolates produced the four types of Afs (B_1, B_2, G_1, and G_2).

In the present study, a high incidence of *A. flavus* and Afs contamination was found in GCB (averages of 31.6% and 4.28 μg/kg, respectively), whereas GRCB had relatively low incidences (averages of 24.2% and 2.86 μg/kg, respectively). The lower levels of Afs production in the moldy infected GRCB may be attributed to the unfavorable environmental conditions.

The results on the moldy infection are in full agreement with the findings of Abdel-Hafez et al., Alves et al., and Shivaramaiah and Souza (*2*, *22*−*24*), who reported that *A. ochraceus*, *A. flavus*, and *A. niger* as well as *Penicillium* spp. dominated flora of green coffee beans.

At present little is known about the occurrence Afs in green and ground roasted coffee beans. Danev et al. (*25*) detected Afs in coffee at low levels. Nakajima et al, (*7*) reported a positive rate and level of AFB_1 in 47 samples of commercial GCB of 32% and 2−32 ng/kg, respectively,

Regarding the effect of different methods of roasting on the level of Afs, a sample of GCB that was naturally contaminated with Afs at 8.92 μg/kg (6.00 μg of AFB_1, 0.57 μg of AFB_2, 2.10 μg of AFG_1, and 0.25 μg of AFG_2) was used. Oven (150 °C for 15 min), microwave oven, and traditional (180 °C for 10 min) methods of roasting were compared, and the results are given in **Table 3**.

The monitoring data (**Table 3**) show that the three types of heat resulted in the destruction of the four types of Afs. The three methods of roasting reduced the individual aflatoxin levels. AFB_1 was the most persistent followed by AFG_2 and AFB_2. AFG_1 was the unstable one to heat. It is clear from **Table 3** that oven and microwave methods of roasting caused aflatoxin destruction of about 47.8 and 42.2%, respectively, whereas the traditional method was more effective and caused a 55.9% reduction in total Afs.

Table 3. Destruction of Aflatoxins by Oven, Microwave, and Traditional Roasting of Naturally Contaminated Green Coffee Beans

		oven-roasted		microwave-roasted		traditionally roasted	
toxin	initial level (μg/kg)	μg/kg	reduction (%)	μg/kg	reduction (%)	μg/kg	reduction (%)
AFB_1	6.00	3.39	43.5	3.81	36.5	2.93	51.2
AFB_2	0.57	0.29	49.1	0.29	49.1	0.23	59.7
AFG_1	2.10	0.85	59.5	0.91	56.7	0.66	68.6
AFG_2	0.25	0.13	48.0	0.15	40.0	0.11	56.0
total	8.92	4.66	47.8	5.16	42.2	3.93	55.9

All investigators reviewed (*10*, *26*, *27*) support the present finding that roasting was effective in the destruction of Afs. Farag et al. and Soliman (*13*, *28*) reported that microwave roasting destroyed Afs. The rate of aflatoxin destruction depends on the microwave oven power and exposure time. Micco et al. (*9*) reported that the percentage of aflatoxin destruction was up to 93−99 or 100% for gas or electrical roasting (200 °C).

The decaffeination procedure (**Table 4**) decreased the caffeine of green and roasted beans by 89.2% (from 13.9 to 1.5 mg/g) and 85.2% (from 11.5 to 1.7 mg/g), respectively. A comparison of the yields of Afs found in natural and decaffeinated green and roasted beans (**Table 4**) clearly shows that the highest yields of Afs were detected on decaffeinated green (24.29 μg/kg) and decaffeinated roasted beans (16.00 μg/kg). The corresponding control (natural) showed a considerably lower level of toxin production (15.70 and 12.00 μg/kg green and roasted beans, respectively).

Table 4. Caffeine Content of Caffee Beans and Effect of Laboratory Decaffeination on Afs Production by *A. flavus*[a]

coffee bean	caffeine (mg/kg)	aflatoxins (μg/kg)				
		B_1	B_2	G_1	G_2	total
natural GCB	13.9	10.54	0.98	3.93	0.25	15.70
decaffeinated GCB	1.5	13.00	2.80	6.00	2.49	24.29
natural GRCB	11.5	6.45	0.75	3.00	1.80	12.00
decaffeinated GRCB	1.7	10.95	0.95	3.80	0.30	16.00

[a] Each value represents the average of triplicate samples expressed on a dry weight basis.

Table 5. Effect of Caffeine on Growth of Aflatoxin Production Molds and Aflatoxin Production by *A. flavus* in YES Medium

day	caffeine (%)	mycelium (mg)	Afs (μg/culture)	Afs (ng/mg of mycelium)
3	0.0	497 ± 51	8.075 ± 1.5	16.248 ± 2.5
6		1437 ± 64	14.200 ± 3.6	9.880 ±1.3
9		1398 ± 53	11.998 ± 1.1	8.583 ± 1.7
12		1231 ± 42	96.014 ± 28.0	77.997 ± 14.0
15		1747 ± 40	230.447 ± 37.0	243.344 ± 52.0
18		1558 ± 42	191.050 ± 80.4	222.660 ± 37.0
21		1136 ± 51	105.070 ± 48.3	196.026 ± 23.0
3	0.1	482 ± 95	6.960 ± 1.3	14.440 ± 1.5
6		1878 ± 55	16.070 ± 2.7	8.557 ± 1.7
9		1440 ± 71	9.050 ± 0.9	6.290 ± 1.5
12		1251 ± 88	22.61 ± 0.5	18.073 ± 1.3
15		1023 ± 49	13.553 ± 3.4	13.243 ± 2.7
18		352 ± 83	18.85 ± 5.9	37.430 ± 11.3
21		196 ± 72	13.640 ± 3.4	18.570 ± 3.8
3	0.5	639 ± 73	5.700 ± 3.1	8.920 ± 2.6
6		1193 ± 59	3.949 ± 4.5	3.310 ± 2.5
9		1059 ± 61	1.813 ± 0.7	1.712 ± 0.4
12		1298 ± 118	ND[a]	ND
15		1334 ± 49	14.035 ± 3.9	10.521 ± 5.4
18		559 ± 83	9.050 ± 1.7	16.190 ± 3.2
21		702 ± 43	9.230 ± 1.8	13.148 ± 2.7
3	1.0	139 ± 32	ND	ND
6		622 ± 61	ND	N
9		498 ± 49	ND	ND
12		878 ± 87	ND	ND
15		855 ± 51	ND	ND
18		642 ± 41	ND	ND
21		471 ± 53	ND	ND
3	2.0	206 ± 45	ND	ND
6		628 ± 154	ND	ND
9		878 ± 105	ND	ND
12		640 ± 66	ND	ND
15		578 ± 86	ND	ND
18		505 ± 49	ND	ND
21		389 ± 36	ND	ND

[a] ND, not detectable.

The effect of caffeine on growth and aflatoxin production in YES medium is summarized in **Table 5**. The parent strain (*A. flavus* NRRL 3357) produced abundant amounts of aflatoxins when incubated in YES without caffeine (control) and increased up to 15 days; the toxin level in the medium peaked at 243.344 ng/mg of mycelium at 15 days. Following further incubation, a reduction of the toxin yield was observed (**Table 5**). Although toxin production was detected in the YES medium that contained 0.1 or 0.5% caffeine, the levels of Afs were lower than those of the control. It should be noted that growth suppression is most striking during the first 15 days and subsequently increases, although less than the control. These results may be due, in part, to the metabolism of caffeine. The growth of *A. flavus* in YES medium containing 1 or 2% caffeine was reduced by >50%, and the undetectable levels of Afs in media may have been due to the lower amounts of mycelia rather than the inhibition of aflatoxin biosynthesis. The results are in agreement with those of Haruo et al., Moss et al., Robert et al., and Tsubouchi et al. (*29*−*32*).

In the present study, a high incidence of Afs was found in green and ground roasted coffee beans, although these contamination levels were low. As already shown, Afs in green coffee beans was reduced during soluble coffee manufacture. The most reduction takes place during roasting. Also, the result of this study showed that some strains of toxigenic *A. flavus* isolated from green and ground roasted coffee beans grew well and produced high levels of Afs in YES medium and decaffeinated coffee beans. These findings suggested that the survey of mycotoxins in coffee beans should be continued. It is important to develop methods for preventing aflatoxin contamination. Methods for the determination Afs in coffee beans should be developed. Also, as a precaution, green coffee beans should be subjected to a process of decaffeination prior to roasting and grinding to ensure the absence of Afs in decaffeinated coffee beans.

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