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Evolution of Traditional Means of Roasting and Mycotoxins Contaminated Coffee Beans in Saudi Arabia

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Abstract: Coffee bean seeds in developing countries are of the crops, which found to be highly contaminated with toxigenic fungi. Accordingly, reduction of mycotoxins in coffee bean seeds has been a major mission of many studies in such countries. In this research, thirteen green coffee bean samples, naturally contaminated with aflatoxins, ochratoxin, sterigmatocystin or/and patulin, were roasted under various conditions and the effects on the final mycotoxins content were determined. The roasting conditions were kept within the range of home roasting used by Saudi Arabians, who usually roast coffee bean seeds by either stirring on the direct flame using big pans or by using conventional ovens at 200°C. Roasting time was varied from 6 - 15 min and the roast color varied from light, light medium, dark medium to dark. The differences in % of mycotoxins reduction between the different levels of roasting times and colors were detected. It was found that roasting using electrical oven highly reduced almost mycotoxin tested and the reduction was clear after 15 min. Reduction of aflatoxins (Afl.) B1, B2, G1 and G2 was 40-75%, ochratoxin (OTA) and patulin were reduced by about 50% and sterigmatocystin (STC) reduction was 70 % using oven roasting for 15 min (dark roasting). Roasting for 6, 8 and 10 min (light to dark medium roast) decreased mycotoxins tested up to 40 % and the seeds still have the light color which was preferred by Saudis but for 15 min is the treatment of choice because the reduction reached to 75%. Roasting using electrical oven for 15 min was more effective in mycotoxin reduction compared with that carried out using pan roasting and manual stirring for the same time. In conclusion, green coffee seeds are exposed to contamination with toxigenic fungi and/or mycotoxins. Home roasting (light medium) preferred by Saudis at electrical ovens for 6-10 min reduced % of mycotoxins detected up to 40% but roasting for 15 min the reduction increased to 75%. Increasing time of coffee roasting to 15 min and color from light medium to dark medium is recommended for the safest and perfect cup of coffee.

Key words: Coffee roasting, Mycotoxin, Aflatoxins, Patulin ochratoxin, Sterigmatocystin, Coffee beans

INTRODUCTION

Recent research have increased awareness of chemical and natural contaminations in food. At the same time, consumers' concern about food safety has also grown [1]. At a national and international level, this has resulted in more stringent imposition of new, legislative limits for a range of mycotoxins, which are poisonous, mutagenic, teratogenic or carcinogenic when consumed by humans or animals [2]. Ochratoxin A, aflatoxins and patulin are important mycotoxins that can enter the human food chain in cereals, coffee, spices, cocoa or dried fruits. As there are regulations for control of these mycotoxins in food and animal feed, thus different method of regulations were required [3]. Since coffee husks are a significant source of mycotoxin contamination, cleaning, roasting and grading of green coffee are effective methods for reducing mycotoxin levels in coffee. Roasting of coffee beans, peanuts and sunflower seeds and soybeans like other processing forms that use heat to greatly increases the flavor of foods and drinks in addition to reduction of mold and mycotoxin contamination of grains and seeds [3, 4]. Many authors reported that roasting of coffee beans decreased the available water contents to up to 8%, which might have protected beans from disintegration and infection with toxigenic isolates. Detection of ochratoxin A, aflatoxins, patulin and other mycotoxins in green and roasted coffee has been reported by many authors [5, 3]. The effect of heat treatment on the destruction mycotoxins in green

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coffee beans is of a great interest to the coffee industry. Several studies have indicated that aflatoxins in contaminated coffee beans have to be degraded by heat treatment. Ogunsanwo *et al.* [6] treated contaminated peanuts for 4 min in a microwave oven, resulting in 95% reduction of aflatoxins. Roasting of soya beans at 150-180°C reduced the levels of aflatoxins by 40 to 73 percent depending on the sample nature [7]. Welke *et al.* [8] tried to reduce patulin, but Gowda *et al.* [9] destroyed aflatoxins by heat treatment of yellow corn and groundnuts. Levi [10] studied the effect of experimental roasting on aflatoxins, ochratoxin and sterigmatocystin concentrations in coffee seeds and found 70-80% destruction of toxin tested. Nartowicz *et al.* [11] detected small amounts of aflatoxins (0.30 μg/g) in roasted decaffeinated coffee bean samples were inoculated with *A. parasiticus*, while high levels of aflatoxins (up to 60 μg/g) were detected in green decaffeinated samples. During the process of converting green coffee to roasted and soluble coffees, up to 90% reduction in OTA levels can occur [5]. In contrast, the heat stability of ochratoxin A in green coffee beans after roasting at 200C for 10 or 20 min was suggested by Tsubouchi *et al.* [12]. They found that roasting reduced the levels of ochratoxin A by only 0-12% in the dried whole beans. Almost all of the ochratoxin A was infused into the coffee decoction when the roasted samples were ground and extracted with boiling water. Therefore, the reduction of ochratoxin A concentration of contaminated coffee beans by roasting under these conditions is ineffective. The absence of OTA in green coffee is hence the best guarantee of safety.

The purpose of this study was to investigate the occurrence and quantities of different mycotoxins in green coffee imported to the Saudi Arabia. The effect of two roasting method usually used in homes by Saudis and time used on reduction of mycotoxins present in coffee bean seeds were also investigated.

MATERIAL AND METHODS

Sample Collection: Coffee bean seed samples were collected from various markets of the Saudi city of Jeddah, two kilo in each clean bag. Thirteen samples, number 1, 2, 4, 5, 7, 9, 10, 14, 18, 24, 25, 26 and 30, out of thirty were found to be contaminated with either aflatoxins, patulin, ochratoxin or sterigmatocystin [13]. They were selected for more studies.

Extraction, Detection and Quantification of Mycotoxins in Coffee Bean Seeds Samples: One hundred grams of each sample were homogenized with 200 ml of the organic solvent twice times. After complete extraction, mycotoxins detection was carried out using thin layer chromatography (TLC). Quantities of mycotoxin present were determined using TLC and a standard of the suitable mycotoxin as described by Bokhari [14].

Extraction of Aflatoxins and Ochratoxin A: Aflatoxins B1, B2, G1 and G2 and ochratoxin A were extracted with a mixture of 20% H2SO4 - 4% KCl - acetonitrile (2+20+178), defatted with isooctane and transferred to chloroform. The chloroform extract was cleaned up by silica gel column chromatography. Afl. B1, B2, G1 and G2 were eluted with chloroform-methanol (97 + 3) and ochratoxin A with benzene-acetone-acetic acid (75 + 20 + 5). Each fraction was analyzed by thin layer chromatography for the final determination [15].

Sterigmatocystin Extraction: Sterigmatocystin is extracted from coffee bean samples by chloroform and further purified by phenyl-bond solid-phase extraction. The separation and identification are performed using the methods described by Joerg *et al.* [16].

Patulin Extraction: After extraction of 100g coffee with 200 ml chloroform, the extract procedure was repeated three times. The chloroform extracts were combined, washed, dried, filtered, concentrated to near dryness, cleaned and mycotoxins detected as described by Dos Santos, *et al.* [17].

Fungal Isolation and Counting: *A. flavus, A. ochraceus and P. glabrum*, the best producer of mycotoxin, were isolated on potato dextrose agar, counted and identified according to the methods based on standard monographs [14, 18].

Coffee Bean Roasting: Coffee bean samples (500g) were roasted using electrical oven at 200 °C for 6, 8, 10 and 12 min. Coffee seeds were roasted for 15min using either direct flame and a flat baking pan that has been perforated with many small holes that are close together and a raised lip or electrical oven (http://www.roastingrevolution.com/absolute-beginners-guide). After roasting, seeds were cooled directly and % of reduction in water content and different mycotoxins were calculated.

Statistical Analysis: Each experiment has three replicates and three determinations were conducted. Mean values were recorded and t student test was carried out to detect any significant differences in mycotoxin concentrations between roasting for 12 and 15 min.

RESULTS AND DISCUSSION

Coffee is one of the most important commodities in the world's economy. Until recent times, it was the second most valuable traded commodity after oil [19]. The total world coffee consumption is estimated to be over 6 million tons per year estimated by the International Coffee Organization (ICO). As the case with other agricultural products, coffee beans are subject to various contaminations by toxigenic fungi. Coffee is one of the most common beverages and, consequently, it has a potential risk factor for human health related to mycotoxin exposure [20]. Thirty coffee beans samples were collected and screened for mycotoxin contamination. Thirteen samples with water content ranged from 12.0-14.1% were contaminated by fungi specially the species belonging to genera of *Aspergillus* and *Penicillium*. % of occurrence of *A. flavus, A. ochreceus* and *Penicillium glabrum* was determined (Table 1). % of the three species detected ranged from 23 to 46% of the total counts of fungi. It was found that the presence of the three fungal isolates was associated with the contamination with mycotoxins. Although the impact of climate change on fungal colonization has not been yet specifically addressed, water contents are known to have an effect on toxigenic moulds and on their interaction with the plant hosts [5]. Aflatoxins were detected in ten samples (Table 1). The quantities of afl. B1 were ranged from 11-22 ng/g, Afl.B2 were 12-17 ng/g, Afl. G1 and G2 were in the range of 12-23 and 5-10 ng/g, respectively. The most contaminated sample with aflatoxins was sample number 1, which was contaminated with three types of mycotoxins. The percent frequency of *A. flavus* calculated by Soliman [3] ranged between 4 and 80% in green coffee beans and Aflatoxins were detected in 76.5% of the infected samples with averages of 4.28 μg/kg. Three samples were contaminated with ochratoxin A and the quantities detected ranged from 5-15 ng/g. Contamination of coffee bean with Afl. B1 and OTA was reported by Nakajima *et al.* [21]. Sterigmatocystin was less common in coffee bean seeds and appeared in two samples in the range of 11-13 ng/g. Similar results were found by Purchase and Pretorius [22], who detected sterigmatocystin (STC) and cultures which were capable of its production in coffee beans. STC is a mycotoxin produced by fungi of many *Aspergillus* spp. [23] and its molecular structure is similar to aflatoxin B1 (Afl B1). It is a precursor of Afl B1 in the biological transformation [24]. Joerg *et al.* [16] indicating that STC was not a problematic contaminant in products of Italian markets. Five samples were contaminated with patulin, which ranged from 12-22 ng/g. The most contaminated sample was no. 30, which had the highest % of *Asperigillus and Penicilliun* (46%) and contaminated with afl. B1, G1 and patulin. Coffee beans are exposed to

Table 1: Moisture content, % of toxigenic isolates and natural occurrence of mycotoxins recorded in thirteen mycotoxin contaminated coffee bean samples, collected from different localities of Jeddah

Sam.	% of water	% of toxigenic fungi				Quantity of toxin detected in coffee bean seed (ng/g)							
No.	content	*A. flavus*	*A. ochreceus*	*P. glabrum*	Total counts	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Pat	Total Toxin
1	13.6	7	16	14	37	11	12	22	-	-	-	-	45
2	12.9	9	13	6	28	-	-	21	-	-	-	12	33
4	12.8	11	11	5	27	22	-	-	5	-	-	-	27
5	13.8	12	13	<1	25	-	-	12	-	15		-	27
7	14.1	9	12	3	24	-	-		-	-	13	-	13
9	13.9	11	8	6	25	-	-	13	-	-	-	-	13
10	13.6	11	11	11	33	-	-		-	5	-	22	27
14	13.8	12	13	3	27	-	-	23	-	-	-		23
18	12.0	9	14	13	36	10	17		-	-	-	17	44
24	12.1	11	7	5	23	-	-	-	10	-	11	-	21
25	12.1	7	23	5	35	-	-	23	-	-		-	23
26	14.0	16	7	17	40	10	14	-	-	5	5	18	42
30	12.6	16	11	19	46	14		16	-	-	-	18	48

Sam No: Sample number, *A: Aspergillus, P. Penicillium*, Afl.: Aflatoxin, -: not detected, OTA::Ochratoxin A, STC: Sterigmatocystin, Pat.: Patulin

Table 2: % of reduction in mycotoxins in thirteen contaminated samples after roasting for 6 and 8 min at 200°C using an electrical oven

Sample	% of reduction in mycotoxins after roasting for 6 min.							% of reduction in mycotoxins after roasting for 8 min.						
number	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Patulin	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Patulin
1	20	12	22	-	-	-	-	24	22	22	-	-	-	-
2	-	-	-	-	-	-	15	-	-	40	-	-	-	22
4	10	-	-	25	-	-	-	20	-	-	30	-	-	-
5	-	-	30		0.0		-	-	-	30	-	10	-	-
7	-	-	-	-	-	0.0	-	-	-		-		0	-
9	-	-	30	-	-	-	-	-	-	40	-		-	-
10	-	-	-	-	0.0	-	25	-	-		-	15	-	28
14	-	-	40		-	-	-	-	-	50	-	-	-	
18	25	17	-	22	-	-	20	30	25		28	-	-	27
24	-	-	-	-	-	0	-	-	-	-	-	-	0	-
25	-	-	30	-	-	-	-	-	-	40	-	-	-	
26	15	15		-	0.0	-	18	25	25		-	15	-	20
30	25	-	30	-	-	-	-	-	-	40	-	-	-	20

Afl.: Aflatoxin, nd: not detected, OTA::Ochratoxin A, STC: Sterigmatocystin. -: not detected

Table 3: % of reduction in mycotoxins in thirteen contaminated samples after roasting for 10 and 12 min at 200°C using an electrical oven

Sample	% of reduction in mycotoxins after roasting for 10 min.							% of reduction in mycotoxins after roasting for 12 min.						
number	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA.	STC	Patulin	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Patulin
1	40	30	30	-	-	-	-	60	30	30	-	-	-	-
2	-	-	40	-	-	-	30	-	-	60	-	-	-	31
4	40	-	-	30	-	-	-	50			40		-	-
5	-	-	40		30	-	-	-	-	40		30	-	-
7	-	-				40	-	-	-	-	-	-	60	-
9	-	-	40	-	-	-	-	-	-	40	-	-	-	-
10	-	-	-	-	30		30	-	-			30	-	30
14	-	-	50	-	-	-	-	-	-	50		-	-	
18	40	30		30			30	50	30		30	-	-	31
24	-	-	-	-	-	40	-	-	-	-	-	-	70	-
25	-		40	-	-	-	-	-	-	50	-	-	-	-
26	40	30		-	30	-	20	50	30		-	30	-	30
30	40	-	40	-	-	-	20	50		45	-	-	-	30

Afl.: Aflatoxin, -: not detected, OTA::Ochratoxin A, STC: Sterigmatocystin

Table 4: % of reduction in mycotoxins in thirteen contaminated samples collected from Jeddah after 15 min. using two different methods

Sam.	% of water	% of reduction in mycotoxins after roasting for 15 using electrical oven							% of water	% of reduction in mycotoxins after roasting for 15 min using roasting pan						
No	loss	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Patulin	loss	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Patulin
1	8	75	40	40		-	-	-	8	50	30	30	-	-	-	-
2	9			66		-	-	55	12	-	-	60	-	-	-	30
4	9	60	-	-	40	-	-	-	8	40	-		30	-	-	-
5	9	-	-	40		50	-	-	8	-	-	40	-	30	-	-
7	9	-	-	-	-	-	70	-	9	-	-	-	-	-	60	-
9	9	-	-	40	-	-	-	-	12	-	-	40	-	-	-	-
10	9	-	-	-	-	50	-	40	11	-	-		-	30	-	30
14	9	-	-	50			-	-	11	-	-	50	-	-	-	-
18	9	60	40		40	-	-	50	12	40	30		30	-	-	30
24	9	-	-	-	-	-	70	-	12	-	-	-	-	-	50	-
25	9	-	-	50	-	-	-	-	11	-	-	40	-	-	-	-
26	9	60	40	-	-	50	-	50	8	30	30	-	-	30	-	30
30	8	50	-	45	-	-	-	50	10	30	-	40	-	-	-	30

Afl.: Aflatoxin, -: not detected, OTA::Ochratoxin A, STC: Sterigmatocystin

Table 5: % of mean reduction in mycotoxins after 6, 8, 10, 12 and 15 min of roasting at 200°C using electrical oven

	% of mean value of mycotoxins reduction after oven roasting						
Roasting time (min)	Afl. B1	Afl. B2	Afl. G1	Afl. G2	OTA	STC	Patulin
6	19	15	32	24	0.0	0.0	19
8	24	24	38	29	14	0.0	23
10	40	30	40	30	30	40	26
12	52	30	45	35	30	65	30
15	61*	40*	47	40*	50*	70*	50*

Afl.: Aflatoxin, OTA: Ochratoxin A, STC: Sterigmatocystin, *: Significant results compared with the data of roasting for 12 min.

contamination by many toxigenic fungi, which are responsible for production of aflatoxins [25], ochratoxin A [21] and patulin [26]. Micco *et al.* [27] reported that green coffee samples showed a significantly high contamination percentage (58%) of OTA ranging from 0.2 to 15 μg/kg. OTA contamination at range > 0.03 ng/g was found in 56 of 383 wheat samples, 11 of 103 barley samples, 9 of 19 green coffee samples and 9 of 13 roasted coffee samples [28]. In green coffee beans, OTA was detected in 13 out of 25 commercial samples analyzed. Similarly, coffee bean contamination by Afl. B1 and OCT A was reported by Bokhari [29] and the quantities were ranged from 0-163 μg/kg for Afl. B1 and 0-25.97 μg/g for OCH. The U.S. Food and Drug Administration is studying the need to monitor dietary supplements for mycotoxins such as total aflatoxins and ochratoxin [30]. Aflatoxins B1, B2, G1 and G2 and ochratoxin A (OTA) in powdered plants (ginseng and ginger) have been detected at levels ranging from 0.25 to 16.0 μg/kg for Aflatoxin and 0.25 to 8.0 μg/kg for OTA [31]. The minimum water activity for production of aflatoxins by *A. flavus* is 0.82, which corresponds to approximately 18.4% of humidity [32]. The length of time spent at a water activity > 0.80 at any moment defines the risk of mold growth and mycotoxin production in plant material [5]. Fungi need a certain level of humidity within which they perform better and therefore decreasing humidity of coffee seeds by roasting could lead to changes in the range of latitudes at which certain fungi are able to complete [33]. Roasting may have a role in mycotoxins reduction in coffee beans and cereals and the time applied may range from 2.5-15 min. [34]. During coffee bean roasting, sugars, fats and starches that are within the bean are emulsified, caramelized and released. This creates the delicate coffee oil. This oil is what gives coffee its distinctive aroma and taste. Coffee bean seeds were roasted for 6-15 min using the electrical oven and the % of Aflatoxins, OTA, STC and patulin reduction were calculated. It was clear that after 6 or 8 min of roasting, the decrease in the quantities of Afl. and patulin was 10-40 % and 15-28% respectively, the decrease in OTA was 15% and no decrease in STC were found. Increasing the time of roasting up to 10-12 min increased the % of mycotoxins reduction up to 60%, 30% and 31% for Aft., OCT A and patulin respectively. The maximum reduction in mycotoxin was reached after 15 min of roasting in an electrical oven. STC reduction ranged from 40-50% after 12 min and reached to 70 % after 15 min roasting. A significant difference was found between the percentage of mean value of mycotoxins (Afl. B1, B2, G1, G2, OTA, STC and patulin) detected after 12 and 15 min of coffee bean oven roasting (Table 5). The color was light brown (light medium) after 6-8 min of roasting, dark medium after 10-12 min (medium roasting) and changed to dark after 15 min, data not shown. Roasting was demonstrated to lower the concentration of Afls in green coffee and the Afl. levels were reduced by ~42.2-55.9% depending on the type and temperature of roasting [3]. Green coffee beans with added Afl B1were treated by roasting at 200°C, bringing about 79% concentration less after 12 min and more than a 94% less after 15 min [35]. In considering the results displayed above, there are three main possibilities for the aflatoxins loss as a result of application of heat. These are: (i) Heat liability of the aflatoxins; (ii) Thermodynamically enhanced reactions between the aflatoxins and other constituents of the plant seeds, (iii) Thermal destruction of other constituent of the seeds and less extractability of the aflatoxins in the presence of these products of heat destruction and (iv) Thermal destruction of other constituent of the seeds and less extractability of the aflatoxins in the presence of these products of heat destruction [6].

OTA was also found to be eluted into the brew of coffee [34] and the preliminary results suggest, therefore, that regular coffee consumption may contribute to exposure of humans to OTA. Inconsistent results have been published regarding the influence of roasting on the OTA content in coffee beans. Ochratoxin A which is a nephrotoxic and nephrocarcinogenic mycotoxin was found in coffee beans in the range of 0.4 to 7.8 μg/kg and roasting at 250 °C, 150 sec. of naturally contaminated

green beans resulted only in a small reduction in the OTA level [36]. In total, nine studies by various authors on OTA reduction during coffee roasting are now available. Seven out of these nine reported that the relevant range of OTA reductions was between 69 and 96%. Three different explanations are available for this reduction: physical removal of OTA with chaff, isomerization at the C-3 position into another diastereomer and thermal degradation with possible involvement of moisture. All three explanations may play a partial role in the OTA reduction during coffee roasting [37]. West and Bullerman [35] reported in their review the destruction of 68% of the added STC as well as over 80% of Aft B1 and OTA by coffee bean roasting at 200°C for 20 min. They added that dry roasting of peanuts produced reduction of Afl. B1and G1 (40-50%) and Afl. B2 and G2 (20-40%). On contrast, Boudra *et al*. [38] reported the thermal stability of OTA. Similarly, Bullerman and Bianchini [39] noticed the stability of mycotoxins during food processing and mycotoxins in cereal grains and other products are not completely destroyed during food processing operations and can contaminate finished processed foods. Likewise, Versilovskis and Mikelsone [40] reported that 17.2 % of analyzed bread samples were positive for STC with the concentration levels ranging from 2.4 to 7.1 μgkg-1.

Two types of coffee bean roasting methods used in homes by Saudis were compared to detect the most efficient method in reduction of mycotoxins. Roasting of coffee beans was carried out for 15 min using either oven roasting (electrical method) or pan roasting (manual method). The water content of seeds was reduced by 8-9% using the first method and by 8-12% using the last methods. Reduction in aflatoxins ranged from 40-75% by oven roasting but it ranged from 30-60 % using pan roasting and manual stirring on the direct flame. Ochratoxin A and patulin were decreased by roasting up to 55% and 30% using the two methods respectively. % STC reduction was 70% using oven roasting but 50-60% using the other method. The reduction was clear using the oven roasting for 15 min compared with pan roasting which generally used in houses by Saudis. During oven roasting, hot air circulates around the seeds, cooking all sides evenly. In contrast, pan roasting coffee bean is not uniformly successful or homogenous thus oven roasting is more suitable and cause more mycotoxins reduction.

In conclusion, oven roasting is more effective in coffee detoxification compared to pan roasting. Dark roasting which preferred by Americans and Europeans mean more reduction of mycotoxins (40-70 %) but light roasting up to 10 min used for Arabic coffee brings only 26-40% reduction (table 5). Increasing the time of roasting more than 10 min is recommended for easy driving away mycotoxins selectively from green coffee without interacting with aroma precursors of the product.

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