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Aflatoxins and Ochratoxin A Content of Stored Yemeni Coffee Beans and Effect of Roasting on Mycotoxin Contamination

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Abstract:

This study was aimed to determine the natural occurrence of ochratoxin A (OTA) and total aflatoxins (B₁, B₂, G₁, and G₂) (AFs) of stored Yemeni green and roasted coffee beans. A total of 50 samples (25 samples each) were collected randomly from different markets in Sana'a city, Yemen during 2013, and quantitative assessment of these two types of mycotoxins was done using ELISA test kits. In addition, the influence of roasting on OTA and AFs contamination of green coffee beans was also investigated using the pan roasting method. Our results showed that all green and roasted coffee bean samples examined were contaminated with OTA, at concentrations ranging from 1.114 to 18.667 ppb and 1.646 to 31.077 ppb respectively. Also, all green and roasted coffee bean samples examined were contaminated with AFs, at concentrations ranging from 14.694 to 27.176 ppb and 14.255 to 23.231 ppb respectively. In addition, the roasting of contaminated green coffee bean samples with different concentrations of OTA and AFs reduced OTA and AFs levels to 86% and 20% from the initial concentration, respectively. These results indicate that there are risks of mycotoxin contamination of stored Yemeni green and roasted coffee beans.

Keywords: Ochratoxin A (OTA), aflatoxins, ELISA kit, green and roasted coffee beans, Yemen.



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INTRODUCTION

Coffee has been the most commercialized food product and most widely consumed beverage in the world for decades (Farah, 2012), being appreciated for its characteristic taste and aroma and, more recently, for its potential beneficial effects on human health (Perrone *et al.*, 2008). Coffee is the second largest traded commodity in the world after petroleum (Machado *et al.*, 2012).

Meanwhile, Yemen is one of the most historic coffee-producing nations; having launched the trade of what has become one of the world's most important agricultural commodities. Yemen mocha coffee is regarded as the most traditional coffee and still one of the world's greatest, uniquely delicious coffees (USAID, 2005).

Mycotoxins are secondary metabolites produced by fungi when they grow on agricultural products before or after harvest or during transportation or storage. The major mycotoxin producing fungi are species within the genera *Aspergillus*, *Fusarium* and *Penicillium* (Al-Jobory *et al.*, 2017; Iqbal *et al.*, 2018; Salem and Ahmad, 2010; Kaushik *et al.*, 2013). Mycotoxins affect food quality, resulting in huge economic losses in addition to being hazardous to consumer health for producing countries. Therefore, mycotoxins are considered an important problem throughout the world in terms of public health, agriculture and economics (Ozer *et al.*, 2012). Among the known mycotoxins, aflatoxins and ochratoxin A are of greatest concern due to their frequent occurrence in foods and their severe effects on animal and human health (Copetti *et al.*, 2012). Detection of ochratoxin A and aflatoxins in green and roasted coffee has been reported by many authors (Nasser, 2008; Soliman, 2002; Viane, 2002).

Ochratoxin A (OTA) is the main mycotoxin that has been detected in coffee (Suarez-Quiroz *et al.*, 2004). It is estimated that 12% of the ochratoxin A consumed by humans corresponds

to coffee beverage (Bandeira *et al.*, 2011). OTA is believed to be produced in nature by three main species of fungi, *Aspergillus ochraceus*, *A. carbonarius* and *Penicillium verrucosum*, with a minor contribution by *A. niger* (Bokhari, 2007). OTA has been shown to possess nephrotoxic, carcinogenic, immunosuppressive and teratogenic properties (Castellanos-Onorio *et al.*, 2011). In 1993, the International Agency for Research on Cancer (IARC), classified OTA as a possible carcinogen (Group 2B) (IARC, 1993). The FAO/WHO Joint Expert Committee on Food Additives and Contaminants (JECFA) established a provisional tolerable weekly intake (PTWI) of 100 ng/kg of body weight (bw) (equivalent to 14 ng/kg body weight/day). Moreover, the European Commission (EC) Scientific Committee on Food (SCF) recommends that level of OTA should be reduced as much as possible, i.e., below 5ng/kg bw/day (JECFA, 1995; Gollücke *et al.*, 2004; Kaushik *et al.*, 2013). The European Union (EU) Legislation set up a maximum level for OTA in roasted coffee (5.0 µg/kg) (EC, 2006), but level for OTA in green coffee is not yet restricted. However, in Europe, there are national limits for OTA in green coffee ranging from 5.0 µg/kg in Finland to 20.0 µg/kg in Greece (EC, 2002, 2006; FAO, 2006). In USA, in contrast, the FDA has not set advisory limits or action levels for ochratoxins in any commodity (Bayman and Baker, 2006).

In addition, aflatoxins (AFs) in green and roasted coffee beans were detected (Soliman, 2002). AFs are produced by *A. flavus* and *A. parasiticus* (Bhatnagar *et al.*, 2006). AFs are the most dangerous mycotoxins because of their highly toxic, carcinogenic, teratogenic, hepatogenic, and mutagenic characteristics (Pariza, 1996; Chu, 1997). The International Agency for Research on Cancer (IARC) working group on carcinogenicity risks for humans has classified aflatoxins as group 1 carcinogens (IARC, 2002). AFs are encountered in a wide range of important agricultural commodities, including cereals, spices, oil seeds, tree nuts, dried fruits and coffee beans (Bokhari and Aly,

2009a; Bhat *et al.*, 2010; Ozer *et al.*, 2012). Most agencies, including the Joint Expert Committee on Food Additives (JECFA) have not set a total daily intake figure. In common with other dietary carcinogens, it is generally accepted that amounts in food should be reduced to the lowest levels that are technologically possible (COE, 2008). The U.S. Food and Drug Administration (FDA) have established limits for aflatoxin in all food at 20 ng/g. The European Commission (EC) has established aflatoxin regulations for almonds and other tree nuts, and has not set advisory limits for aflatoxins in coffee beans (Whitaker and Slate, 2006).

All coffee is roasted before being consumed. Roasting is an important step that has a great degree of influence on the taste of the final product (Kummer, 2003). Previous studies on the issue provide evidence that OTA levels decrease during the roasting process of coffee beans (Vanessa and Ana, 2013). As well several studies have indicated that aflatoxins in contaminated coffee beans have to be degraded by heat treatment (Bokhari and Aly, 2009b), but on the other hand, it is known that some mycotoxins such as aflatoxins and ochratoxin A maintain a certain stability during most thermal food processing stage (Copetti *et al.*, 2012). The aim of this study was to assess the natural occurrence of ochratoxin A (OTA) and total aflatoxin (B1, B2, G1 and G2) (AFs) of stored Yemeni green and roasted coffee beans and determine the effect of roasting on mycotoxin content.

MATERIAL AND METHODS

Coffee bean samples collection

A total of 50 samples of stored Yemeni coffee beans including 25 green and 25 roasted (of the same type of the green ones) coffee bean samples were randomly collected from different markets and shops at Sana'a city, Republic of Yemen, during 2013. The weight of each sample was 250 g. Each sample was put in a sterile

polyethylene bag, sealed, put in another bag, and transferred to the Microbiology laboratory, Biology Department, Faculty of Science, Sana'a University, Yemen and kept at 4°C until mycotoxins analysis.

Detection and assay of mycotoxins in coffee samples

The coffee bean samples were analyzed to determine their content of ochratoxin A and total aflatoxin by enzyme-linked immunosorbent assay (ELISA) technique. Commercial ELISA Kits were purchased from Helica-United States: total Aflatoxins assay (CAT. NO. 941AFL01M-96) and Ochratoxin A assay (CAT. NO. 961OCH01COF-96). All sample preparation and test procedures were conducted according to the manufacturer's instructions. The range of these two kits was 1-20 ppb.

Effect of roasting on ochratoxin A and total aflatoxin levels

After ochratoxin A and aflatoxins quantification of 25 samples of stored green coffee beans, 20 samples of green coffee beans were selected from the 25 samples contaminated with ochratoxin A (from 2.0 ppb to 18.6 ppb), and 20 samples of green coffee beans were selected out of 25 samples contaminated with aflatoxins (ranging from 16.4 ppb to 23.2 ppb) for roasting test to determine whether the mycotoxins levels will be affected by roasting.

Roasting process

The contaminated green coffee bean samples (100g) were roasted for 15 minutes using direct flame and a flat baking pan that has a raised lip. After roasting, coffee beans were packed in polyethylene plastic bags and kept in a freezer (-18°C) (Bokari and Aly, 2009b), and thereafter analyzed for ochratoxin A and aflatoxins in roasted beans using ELISA kits as mentioned previously and the percentage of reduction in mycotoxins were calculated.

RESULTS AND DISCUSSION

Our results showed that, all 50 samples examined were contaminated (100%) with ochratoxin A and aflatoxins (Table 1). This result is in agreement with Jorgensen (1998) who detected OTA in all of 11 roasted coffee beans samples. Pardo *et al.* (2004) detected OTA in all 57 green coffee samples from different origins. Bokhary and Aly (2009a) reported that percentage of contamination with Aflatoxins B₁,

B₂, G₁ and G₂ for 13 samples contaminated with mycotoxins were 23.3%, 16.6%, 10 and 6.6%, respectively. Mycotoxins contamination is one of the most relevant and worrisome problems concerning food and feed safety. It can produce a variety of toxic acute and chronic effects in human (Maaroufi *et al.*, 1994) and animals (Van der Stegen *et al.*, 2000; Aydin *et al.*, 2003). Among mycotoxins, AFs and OTA are the major concern, given their high occurrence and toxicity (Ozer *et al.*, 2012).

Table 1. Ochratoxin A and total aflatoxin content of green and roasted coffee bean samples.

Green coffee bean samples			Roasted coffee bean samples		
Sample No.	OTA (ppb) *	AFs (ppb)	Sample No.	OTA (ppb) *	AFs (ppb)
1	2.242	16.498	2	2.469	17.437
3	4.783	21.598	4	2.421	16.839
5	1.114	17.700	6	1.646	14.255
7	2.004	21.849	8	2.170	20.085
9	2.402	22.013	10	2.962	20.307
11	4.350	22.096	12	4.853	20.493
13	3.882	23.818	14	2.405	21.603
15	7.321	25.861	16	3.679	22.281
17	2.897	22.528	18	2.395	20.671
19	3.734	22.528	20	7.263	19.806
21	4.147	26.722	22	3.601	22.783
23	5.353	23.260	24	31.077	21.081
25	4.964	21.444	26	4.438	21.431
27	3.930	20.996	28	3.851	19.819
29	4.824	19.690	30	4.492	19.525
31	8.334	14.694	32	4.823	19.380
33	4.582	26.239	34	4.556	23.066
35	12.210	23.466	36	6.338	22.267
37	6.587	24.169	38	9.809	21.291
39	14.982	24.789	40	14.976	22.815
41	18.667	25.741	42	18.869	20.333
43	9.681	26.817	44	5.404	22.723
45	15.321	26.326	46	15.722	21.989
47	5.999	27.176	48	5.074	23.231
49	16.734	24.876	50	15.764	22.370

*= Part per billion.

Table 2. Percentage of contamination and the mean of mycotoxins concentrations (ppb) in tested coffee bean samples according to type of sample.

Type of sample	No. of tested samples	No. of contaminated samples	% of Cont.	OTA levels (ppb)	Conc.	Mean of OTA Conc. (ppb)	AFs levels (ppb)	Conc.	Mean of AFs Conc. (ppb)
Green	25	25	100	1.114 – 18.667		6.842	14.694 – 27.176		22.916
Roasted	25	25	100	1.646 – 31.077		7.242	14.255 – 23.231		20.715
Total	50	50	100	1.114 – 31.077		7.042	14.255 – 27.176		21.816

Conc.= concentration; Cont.= contamination

Our results in Table (1 & 2) also showed that the green coffee bean samples were contaminated with OTA, at concentrations ranging from 1.114 to 18.667ppb, with an average of 6.842 ppb, while roasted coffee bean samples were contaminated with OTA, at concentrations ranging from 1.646 to 31.077 ppb, with an average of 7.242 ppb. The mean cumulative of OTA concentrations for all the tested samples was 7.042 ppb. The presence of OTA in coffee beans has been reported by several authors but in different concentrations, reported levels have been from 0.9 to 19.4 µg/kg (Diaz *et al.*, 2004), 1.3 to 31.5 ng/g (Pardo *et al.*, 2004), 0.2 to 62 µg/kg (Heilmann *et al.*, 1999), 0-48 µg/kg (Romani *et al.*, 2000), 0.3 and 6.5 µg/kg (Leoni *et al.*, 2000) and 0.314 to 3.443 ng/g (Nogaim and Gowri, 2013). Our results in Figure (1) indicated that 44% of green coffee bean samples and 40% of roasted coffee bean samples had OTA levels exceeding European Limits, 5 µg/kg (Notice: These is no Yemeni limit standard for OTA).

A variety of reasons can contribute to the contamination and the production of OTA by toxigenic molds and detection high levels of OTA in coffee bean samples. This might be probably due to variety of environment conditions such as climate, length of storage and transportation (Nakajima *et al.*, 1997). Vasanthi and Bhat (2000) reported that coffee seeds are liable to mold attack, especially when they are not dried to a safe moisture level (11%). The same authors reported that mold damage occurs

during post-harvest stages due to improper drying at the drying yard or during storage of highly moist seeds and leads to OTA production. It is obvious from the current data that the analyzed roasted coffee bean samples showed relatively higher concentrations of OTA than the green ones. This might be probably due to re-contamination of stored roasted coffee beans in shop (contained OTA before roasting) during storage which encourages the growth of toxigenic strains and leads to comparatively higher OTA production as well.

The present investigation also clearly demonstrated coffee bean samples tested were contaminated with AFs (B₁, B₂, G₁ and G₂), at concentrations ranging from 14.694 to 27.176 ppb, with an average of 22.916 ppb for green coffee bean samples, while roasted coffee bean samples were contaminated with AFs, at concentrations ranging from 14.255 to 23.231 ppb, with an average of 20.715 ppb. The mean cumulative of total aflatoxins concentrations for all the tested samples was 21.816 ppb (Table 1 & 2). Various studies reported different concentrations of AFs in coffee beans, Soliman (2002) mentioned that average of 4.28 and 2.85 µg/kg AFs were detected in green coffee beans and ground roasted coffee beans, respectively. Nasser (2008) published that 11 samples of Yemeni coffee beans collected from different markets in El-Riyadh, Saudi Arabia were contaminated with Aflatoxin B₁ ranged from 110-600 µg/kg, Aflatoxin B₂ ranged from 360-600 µg/kg, Aflatoxin G₁ and Aflatoxin G₂ were 600

$\mu\text{g}/\text{kg}$, and Bokhari and Aly (2009b) reported that 10 out of 13 green coffee bean samples were contaminated with Aflatoxin B₁, B₂, G₁ and G₂ at levels ranging from 11-22, 12-17, 12-23 and 5-10 ng/g, respectively. Our results in Figure (1) showed that 84% of green coffee and 72% of roasted coffee bean samples contained more than 20 $\mu\text{g}/\text{kg}$ which is currently the limit being proposed by FDA and Yemeni standard limits for Aflatoxins level in food, which could be explained according the suggestion of (Hill *et al.*, 1985) who reported that the highest concentration of AFs are produced as a result of post-harvest spoilage of commodities stored under warm moist conditions, significant concentrations may also be produced in the field before harvest. In addition, the toxigenic strains of *Aspergillus flavus* are distributed worldwide in soil and air and have been reported to contaminate a variety of food and feeds (Bilgrami, 1984; Mahmoud, 1993).

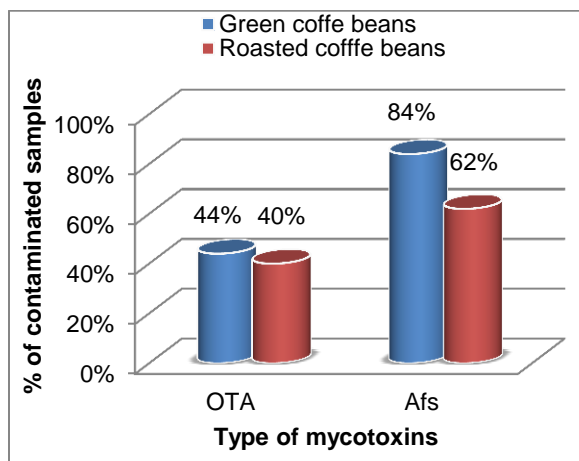


Fig. 1. Percentage of samples contained mycotoxins concentration higher than limits according to EU and FDA legislation.

In addition, data in Table (3) and Figure (2) showed a large range of OTA reduction levels from 6% to 86% with mean 40%. Whereas, the highest reduction was in sample

No. 39 and the lowest reduction in samples No. 3, 15, 21, 33, 43 and 47.

Our results also showed that the relevant range of AFs reduction was between 1% and 20 % with mean 9%. Whereas, the maximum reduction in level was in sample No.45 and the minimum reduction in samples No. 9, 19 and 25. In this concern, contradictory reports have been published in the literature on the reduction of OTA during the roasting of coffee beans, where some authors have reported only a slight reduction of OTA content after coffee beans roasting. For example, Tsubouchi *et al.* (1987) who found that roasting reduced the levels of ochratoxin A by only 0-12% in the dried whole beans and Nehad *et al.* (2005) who reported that roasting reduced OTA by 31%. While other authors have reported high destruction for OTA after roasting such as Blanc *et al.* (1998) published that over half of OTA is destroyed during roasting and Romani *et al.* (2003) obtained reduction of more than 90 % of OTA.

In considering the results described above, there are four main possibilities for the ochratoxin A loss as a result of application of heat. These are: (i) Physical removal of OTA with chaff; (ii) Isomerization at the C-3 position into another diastereomer (Van der Stegen *et al.*, 2001) (iii) The explanation of OTA may be due to direct effects of heat as destroying the side groups in OTA structure as OH or O or indirect effects on water content to produce free radical to interact with side groups in OTA (Zaied *et al.*, 2012) and (iv) The inhomogeneity of OTA distribution within the coffee samples (Studer-Rohr *et al.*, 1994).

Our results of AFs reduction is similar to that reported by Abu Zinadah (2007) found that roasting for 8 minutes at electric oven was sufficient to reduce the quantities of the tested aflatoxin B₁ and G₁ by 24.3% and 21.4% respectively, and contrast to Bokhari and Aly (2009b) who reported that reduction in aflatoxins ranged from 30-60% using pan roasting for coffee beans. The slight reduction of aflatoxins could be due to three reasons: (i) Heat stability

of the aflatoxins (Weidenbörner, 2001), (ii) Thermodynamically enhanced reactions between the aflatoxins and other constituents of the plant seeds (Ogunsanwo *et al.*, 2004) and

(iii) Dry heating ineffective in destroying aflatoxins (Shinha and Bhatnagar, 1998).

Table 3. Percentage of reduction in mycotoxins (OTA & AFs) in contaminated green coffee bean samples after roasting.

Sample No.	% of reduction in mycotoxins		Sample No.	% of reduction in mycotoxins	
	OTA	AFs		OTA	AFs
3	6 %	13 %	27	46 %	-
7	-	2 %	31	58 %	-
9	-	1 %	33	6 %	13 %
11	21 %	7 %	35	75 %	5 %
13	53 %	8 %	37	51 %	10 %
15	6 %	13 %	39	86 %	13 %
17	57 %	4 %	41	70 %	12 %
19	44 %	1 %	43	6 %	15 %
21	6 %	16 %	45	72 %	20 %
23	35 %	4 %	47	6 %	19 %
25	38 %	1 %	49	57 %	9 %

- = Not examined.

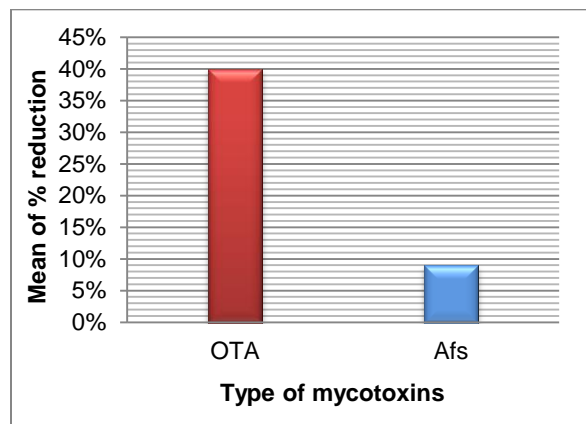


Fig. 2. The mean of percentage reduction in two types of mycotoxins after roasting.

CONCLUSION

It can conclude that, the most Yemeni green and roasted coffee beans have high levels of mycotoxins (OTA and AFs) contamination, especially when it is stored in less hygienic conditions, and stored coffee produced in Yemen presents risk by human exposure to OTA

or AFs through its consumption. In addition, roasting by pan method in homes do not completely eliminate OTA and AFs.

CONFLICT OF INTEREST

All the authors have declared that no conflict of interest exists.

REFERENCES

- Abu Zinadah, G.M., 2007. Mycotoxins of some Toxigenic Aspergilli and Penicilli Strains on Coffee Seeds in Saudi Arabia. M.Sc. Thesis, Faculty of Sciences, King Abdulaziz University, Saudi Arabia.
- Al-Jobory, H.J., Mahmoud, A.L.E., Al-Mahdi, A.Y., 2017. Natural Occurrence of *Fusarium* Mycotoxins (Fumonisin, Zearalenone and T-2 toxin) in Corn for Human Consumption in Yemen. PSM Microbiol., 2(2): 41-46.

- Aydin, G., Ozcelik, N., Cicek, E., Soyoz, M., 2003. Histopathologic Changes in Liver and Renal Tissues Induced by Ochratoxin A and Melatonin in Rats. *Human Exp. Toxicol.*, 22(7): 383-391.
- Bandeira, R.D.C.C., Uekane, T.M., Cunha, C.P., Costa, D.A., Cruz, M.H.C., Godoy, R.L.O., Cunha, V.S., 2011. Optimization of Electrospray Technique Coupled to Mass Spectrometry for the Determination of Ochratoxin A in Coffee. *Rev. de Ci. Vida. Seropédica.*, 31(2): 45-55.
- Bayman, P., Baker, J.L., 2006. Ochratoxins: A Global Perspective. *Mycopathologia.*, 162: 215-223.
- Bhat, R., Rai, R.V., Karim, A.A., 2010. Mycotoxins in Food and Feed: Present Status and Future Concerns. *Compr. Rev. Food Sci. Food Safety.*, 9: 57-81.
- Bhatnagar, D., Cary, J.W., Ehrlich, K., Yu, J., Cleveland, T.E., 2006. Understanding the Genetics of Regulation of Aflatoxin Production and *Aspergillus flavus* Development. *Mycopathologia.*, 162: 155-166.
- Bilgrami, K.S., 1984. Mycotoxin in Food. *J. Ind. Bot. Soc.*, 63: 976-977.
- Blanc, M., Pittet, A., Munoz-Box, R., Viani, R., 1998. Behavior of Ochratoxin A during Green Coffee Roasting and Soluble Coffee Manufacture. *J. Agric. Food Chem.*, 46: 673-675.
- Bokhari, F.M., 2007. Mycotoxins and Toxigenic Fungi in Arabic Coffee Beans in Saudi Arabia. *Adv. Biol. Res.*, 1(1-2): 56-66.
- Bokhari, F.M., Aly, M.M., 2009a. Trials Towards Reduction of Fungal Growth and Aflatoxin G1 Production in Arabic Coffee Using Different Additives. *Afr. J. Food Sci.*, 3(3): 068-076.
- Bokhari, F.M., Aly, M.M., 2009b. Evolution of Traditional Means of Roasting and Mycotoxins Contaminated Coffee Beans in Saudi Arabia. *Adv. Biol. Res.*, 3(3-4): 71-78.
- Castellanos-Onorio, O., Gonzalez-Rios, O., Guyot, B., Fontana, T.A., Guiraud, J.P., Schorr-Galindo, S., Durand, N., Suarez-Quiroz, M., 2011. Effect of Two Different Roasting Techniques on the Ochratoxin A (OTA) Reduction in Coffee Beans (*Coffea arabica*). *Food Control.*, 22: 1184-1188.
- Chu, F.S., 1997. Mode of Action of Mycotoxins and Related Compounds. *Adv. Appl. Microbiol.*, 40: 352-357.
- COE. (Council of Europe Publishing Editions du Conseil de l'Europe), 2008. Guidelines for Risk Assessment, Prevention and Management: Health Risks and Safety Hazards Related to Pest Organisms in Stored Products. Council Europe Publishing, pp. 90.
- Copetti, M.V., Iamanaka, B.T., Pereira, J.L., Lemes, D.P., Nakano, F., 2012. Co-occurrence of Ochratoxin A and Aflatoxins in Chocolate Marketed in Brazil. *Food Control.*, 26: 36-41.
- Diaz, G.J., Ariza, D., Perilla, N.S., 2004. Method Validation for the Determination of Ochratoxin A in Green and Soluble Coffee by Immunoaffinity Column Cleanup and Liquid Chromatography. *Mycotoxin Res.*, 20: 59-67.
- EC. (European Commission), 2002. Task 3.2.7 "Assessment of Dietary Intake of Ochratoxin A by the Population of EU Member States". Rome, Italy, January.
- EC. (European Commission), 2006. Regulation (EC) 1881/2006 of 19/12/2006. *Official J. Eu. Commun.*, L 364, pp. 5-24.
- FAO. (Food and Agricultural Organization), 2006. Reducing Ochratoxin A in Coffee. Available at: <http://www.coffee-ota.org/frq.asp>.
- Farah, A., 2012. Coffee Constituents. In: *Coffee: Emerging Health Effects and Disease Prevention*, First edition. Ed. Chu, Y.-F., Blackwell Publishing Ltd., pp. 21-58.
- Gollücke, A.P.B., Taniwaki, M.H., Tavares, D.Q., 2004. Survey on Ochratoxin A in Brazilian Green Coffee Destined for Exports.

- Ciência e Tecnologia de Alimentos, Campinas, 24(4): 641-645.
- Heilmann, W., Rehfeldt, A.G., Rotzoll, F., 1999. Behaviour and Reduction Ochratoxin A in Green Coffee Beans in Response to Various Processing Methods. Eur. Food Res. Technol., 209: 297-300.
- Hill, R.A., Wilson, D.M., McMillan, W.W., Widstrom, N.W., Cole, R.J., Sanders, T.H., Andblankenship, P.D., 1985. Ecology of the *Aspergillus flavus* Group and Aflatoxin Formation in Maize and Groundnut. In: Trichothecenes and Other Mycotoxins. Ed. Lacey, J., Chichester., John Wiley, pp. 79-95.
- IARC. (International Agency for Research on Cancer), 1993. Ochratoxin A. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, International Agency for Research on Cancer: Lyon, France, 56: 489-571.
- IARC. (International Agency for Research on Cancer), 2002. Aflatoxins. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, some Traditional Herbal Medicines, some Mycotoxins, Naphthalene and Styrene, International Agency for Research on Cancer: Lyon, France, 82: 171-366.
- Iqbal, M.N, Ashraf, A., Ling, S., Wang, S., 2018. *In Vitro* Improved Production of Monoclonal Antibody against Zearalenone in Supplemented Cell Culture Media. PSM Biol. Res., 3(3): 106-110.
- JECFA. (Joint Expert Committee on Food Additive and Contaminants), 1995. Evaluation of Certain Food Additive and Contaminants. In: Technical Report Series n. 859, Geneva: Joint FAO/WHO Expert Committee on Food Additives.
- Jorgensen, K., 1998. Survey of pork, poultry, coffee, bear and pulses for ochratoxin A. Food Addit. Contam., 15: 550.
- Kaushik, A., Arya, S.K., Vasudev, A., Bhansali, S., 2013. Recent Advances in Detection of Ochratoxin-A. Open J. Appl. Biosensor., 2: 1-11.
- Kummer, C., 2003. The Joy of Coffee: The Essential Guide to Buying, Brewing, and Enjoying. Houghton Mifflin, Boston, pp. 37.
- Leoni, L.A.B., Soares, L.M.V., Liveira, L.P.C., 2000. Ochratoxin A in Brazilian Roasted and Instant Coffees. Food Addit. Contam., 17: 867-870.
- Maaroufi, K., Pfohl-Lesszkowicz, A., Achour, A., May, M., Grosse, Y., Hammami, M., Ellouz, F., Creppy, E.E., Bacha, H., Nchartoxin, A., 1994. Ochratoxin A Genotoxicity, Relation to Renal Tumors. Arch. de La Institute Pasteur de Tunis., 71(1-2): 21-31.
- Machado, E.M.S., Rodriguez-Jasso, R.M., Teixeira J.A., Mussatto, S.I., 2012. Growth of Fungal Strains on Coffee Industry Residues with Removal of Polyphenolic Compounds. Biochem. Engin. J., 60: 87-90.
- Mahmoud, A.L.E., 1993. Toxigenic Fungi and Mycotoxic Content in Poultry Feed stuff Ingredients. J. Appl. Microbiol., 84: 62-76.
- Nakajima, M., Tsubouchi, H., Miyabe, M., Ueno, V., 1997. Survey of Aflatoxin B1 and Ochratoxin A in Commercial Green Coffee Beans by HPLC Linked with Immunoaffinity Chromatography. Food Agric. Immunol., 9(2): 77-83.
- Nasser, L.A., 2008. Mycoflora, Mycotoxins, Bacteriological Analysis and Molecular Assay of some Bacterial Species from Coffee Beans in Saudi Arabia. Bulletin of Pharmaceutical Sciences, Assiut University, 31: 345-373.
- Nehad, E.A., Farag, M.M., Kawther, M.S., Abdel-Samad, A.K.M., Naguib, K., 2005. Stability of Ochratoxin A (OTA) during Processing and Decaffeination in Commercial Roasted Coffee Beans. Food Additives and Contaminants, 22: 761-767. In cited: Makun, H.A. (2013): Mycotoxin and Food Safety in Developing Countries. INTECH Publishing, pp. 182.

- Nogaim, Q.A., Gowri, P.M., 2013. Determination of Ochratoxin A in Yemeni Green Coffee. *Scholars Acad. J. Biosci.*, 1(6): 253-262.
- Ogunsanwo, B.M., Faboya, O.O.P., Idowu, O.R., Lawal, O.S., Bankole, S.A., 2004. Effect of Roasting on the Aflatoxin Contents of Nigerian Peanut Seeds. *Afr. J. Biotechnol.*, 3(9): 451-455.
- Ozer, H., OktayBasegmez, H.I., Ozay, G., 2012. Mycotoxin Risks and Toxigenic Fungi in Date, Prune and Dried Apricot among Mediterranean Crops. *Phytopathol. Mediterr.*, 51(1): 148-157.
- Pardo, E., Marin, S., Ramos, A.J., Sanchis, V., 2004. Occurrence of Ochratoxigenic Fungi and Ochratoxin A in Green Coffee from Different Origins. *Food Sci. Technol. Int.*, 10: 45-49.
- Pariza, W.M., 1996. Toxic Substances. In: *Food Chemistry*. Ed. Fennema, O. R., 3rd Edition, Marcel Dekker, New York, pp. 825-841.
- Perrone, D., Donangelo, C.M., Farah, A., 2008. Fast Simultaneous Analysis of Caffeine, Trigonelline, Nicotinic Acid and Sucrose in Coffee by Liquid Chromatography-Mass Spectrometry. *Food Chem.*, 110: 1030-1035.
- Romani, S., Sacchetti, G., Chaves Lopez, C., Pinnavaia, G.G., Dalla Rosa, M., 2000. Screening on the Occurrence of Ochratoxin A in Green Coffee Beans of Different Origins and Types. *J. Agric. Food Chem.*, 48: 3616-3619.
- Romani, S., Pinnavaia, G.G., Roas, M.D., 2003. Influence of Roasting Levels on Ochratoxin A Content in Coffee. *J. Agric. Food Chem.*, 51: 5168-5171.
- Salem, N.M., Ahmed, R., 2010. Mycotoxins in Food from Jordan: Preliminary Survey. *Food Control.*, 21: 1099-1103.
- Shinha, K.K., Bhatnagar, D., 1998. *Mycotoxins in Agriculture and Food Safety*. CRC Press, pp. 387.
- Soliman, K.M., 2002. Incidence, Level and Behavior of Aflatoxins during Coffee Bean Roasting and Decaffeination. *J. Agric. Food Chem.*, 50(25): 7477-7481.
- Studer-Rohr, I., Dietrich, D.R., Schlatter, J., Schlatter, C., 1994. Ochratoxin A and Coffee. *Mitteilungen Aus Dem Gebiete Der Lebensmittelunt. Erziehung and Hyg.*, 85: 719-727.
- Suárez-Quiroz, M., González-Rios, O., Barel, M., Guyot, B., Schorr-Galindo, S., Guiraud, J., 2004. Study of Ochratoxin A-Producing Strains in Coffee Processing. *Int. J. Food Sci. Technol.*, 39: 501-507.
- Tsubouchi, H., Yamamoto, K., Hisada, K., Sakabe, Y., Udagawa, S., 1987. Effect of Roasting on Ochratoxin A Level in Green Coffee Beans Inoculated with *Aspergillus ochraceus*. *Mycopathologia.*, 97(2): 111-115.
- USAID. (United States Agency for International Development), 2005. *Moving Yemen Coffee Forward. Assessment of the Coffee Industry in Yemen to Sustainably Improve Incomes and Expand Trad.* This Publication Was Produced for Review by United State Agency for International Development, It was Prepared by ARD, Inc. December, 2005.
- Van der Stegen, G.H., Essens, P.J., Van der Lijn, J., 2000. Effects of Roasting Conditions on Reduction of Ochratoxin A in Coffee. *J. Agric. Food Chem.*, 49(10): 473-475.
- Van der Stegen, G.H.D., Essens, P.J.M., Van der Lijn, J., 2001. Effects of Roasting Conditions on Reduction of Ochratoxin A in Coffee. *Journal of Agricultural and Food Chemistry*, 49: 4713-4715. In cited: Makun, H.A. (2013): *Mycotoxin and Food Safety in Developing Countries*. INTECH Publishing, pp. 182.
- Vanesa, D., Ana, P., 2013. Occurance of Ochratoxin A in Coffee Beans, Ground Roasted Coffee and Soluble Coffee and Method Validation. *Food Control.*, 30: 675-678.
- Vasanthi, S., Baht, R.V., 2000. Ochratoxin in Indian Coffee and Its Implication to International Trade. *J. Nutr.*, 21: 718-721.

- Viane, R., 2002. Effect of Processing on Ochratoxin A (OTA) Content of Coffee. In: Mycotoxins and Food Safety. Eds. Devries, J.W., Truchsess, M.W. and Jackson, L.S, Springer, vol. 504, pp. 189-193.
- Weidenbörner, M., 2001. Encyclopedia of Food Mycotoxins. Springer Science & Business Media, pp. 15.
- Whitaker, T.B., Slate, A.B., 2006. Sampling Almonds for Aflatoxin, Part I: Estimation of Uncertainty Associated with Sampling, Sample Preparation, and Analysis. J. AOAC Int., 89(4): 1027-1034.
- Zaied, S.A.F., Aly, S.E., EL-Sideek, L.E., Alkhalifah, D.H.M., 2012. Electron Spin Resonance (ESR) Studies on-Irradiated and Roasting Coffee Beans during Decontaminated of OTA. Austr. J. Basic Appl. Sci., 6(3): 741-750.