UDC 614.31: 543.51: 543.544.5.068.7: 543.64
DOI: 10.21668/health.risk/2022.2.06.eng

Read online

Research article

MYCOTOXINS IN COFFEE AND CHICORY: FROM REGULATED TO EMERGENT

I.B. Sedova, M.G. Kiseleva, Z.A. Chalyy

Federal Research Centre of Nutrition, Biotechnology and Food Safety, 2/14 Ustinskiy proezd, Moscow, 109240, Russian Federation

Coffee is a daily basic food product for many people all over the world. In Russia and some European countries, people who try to pursue healthy lifestyle often prefer chicory as a substitute to coffee. Our research goal was to evaluate occurrence of Aspergillus, Penicillium, Fusarium and Alternaria secondary metabolites in coffee and chicory distributed on the RF market.

29 mycotoxins were determined in 48 samples of coffee and chicory using ultra high-performance liquid chromatography coupled with tandem mass-spectrometric detection (UHPLC-MS/MS).

The range of analyzed contaminants included regulated mycotoxins (aflatoxins, ochratoxin A, deoxynivalenol, fumonisins, T-2 toxin, and zearalenone), their derivatives and structural analogs (A and B trichothecenes), Alternaria metabolites (alternariol, its methyl ether, altenuene, tentoxin), citrinin and several emergent mycotoxins (citreoviridin, cyclopiazonic and mycophenolic acids, enniatins, beauvericin).

To the best of our knowledge, the present study is the first to report results indicating that unregulated emergent mycotoxins occur in the examined products. Chicory samples contained beauvericin (9 of 16 samples, the contents varied from 2.4 to 1173 µg/kg) and enniatin B (6 of 16 samples, 2.8–1109 µg/kg). Green and roasted coffee samples contained mycophenolic acid (11 of 20 samples, 23.5–58.3 µg/kg; 3 of 12 samples, 155.7–712.2 µg/kg accordingly). Several samples were contaminated with aflatoxins, ochratoxin A and fumonisin B2. Their contents in the examined samples did not exceed maximum levels; however, their occurrence indicates a potential health risk for consumers. This requires hygienic assessment and monitoring of these products with the focus on their contamination not only with regulated aflatoxin B1 and ochratoxin A but also with other potentially hazardous mycotoxins.

Keywords: *mycotoxins, emergent mycotoxins, coffee, chicory, ochratoxin A, aflatoxins, contamination, UHPLC-MS/MS.*

Coffee is globally one of the most widely consumed natural beverages. It accounts for 75 % of non-alcoholic drinks' consumption [1]. In the Russian Federation in 2019, coffee consumption exceeded tea consumption by 12 %. Arabica *(Coffea arabica)* and Robusta *(Coffea canephora* var. *Robusta)* are the most widely spread coffee varieties [2]. Besides, there are 125 other varieties including Liberica *(Coffea liberica, Coffea excelsa),* which is grown in South-East Asia and is well-known for its bitter taste; Eugenioides *(Coffea eugenioides)*, grown in Ethiopia; and Cameroon coffee *(Coffea charrieriana)* grown in Cameroon [3]. Health benefits of green coffee are well known at present and it results in growing consumption of beverages based on it [4]. Chicory (*Dorema aucheri*) belongs to *Asteraceae* family and is used as an alternative to coffee. Its taste reminds coffee, but it doesn't contain caffeine.

As any other agricultural products, coffee and chicory can be contaminated with substantial amounts of mold fungi (microfungi) including their toxigenic species. Coffee may be contaminated at any stage in its production, prior to and after harvesting. Coffee tree, being a warm-climate plant, grows only in tropical and subtropical climate. Plants in countries with hot and humid climate are most often

Irina B. Sedova – Candidate of Biological Sciences, Senior Researcher at the Laboratory for Enzymology of Nutrition (e-mail: isedova@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-6011-4515).

Mariya G. Kiseleva – Candidate of Chemical Sciences, Senior Researcher at the Laboratory for Enzymology of Nutrition (e-mail: mg_kiseleva@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0003-1057-0886).

Zakhar A. Chalyy – Junior Researcher at the Laboratory for Enzymology of Nutrition (e-mail: brew@ion.ru; tel.: +7 (495) 698-53-65; ORCID: https://orcid.org/0000-0002-9371-8163).

contaminated with microfungi from *Aspergillus* and *Penicillium* genus that produce the most hazardous mycotoxins (MTs) such as aflatoxins (AFL) and ochratoxin A (OTA) [5, 6]. It is known that fungi from *Aspergillus* species such as *A. carbonarius, A. niger, A. ochraceus* and *A. westerdijkiae* are the main OTA producers in tropical and subtropical coffee plantations and *Penicillium verruculosum, P. brevicompactum, P. crustosum, P. olsonii* и *P.oxalicum* in temperate regions [7, 8]. The capacity of an OTA-producing strains to contaminate the coffee beans depends on several factors such as climatic conditions, storage and transportation, and also processing (wet and dry processing) conditions [9]. Fungi from *Fusarium* genus are also considered natural contaminants in coffee [5].

Most published reports related to coffee bean MT contamination are devoted to its contamination with OTA. There is information about contamination of coffee from Brazil, Vietnam, Guatemala, Indonesia, China, Cote-d'Ivoire [10], the South Korea, Malaysia, Taiwan, the Philippines and Ethiopia with the toxin [11–15]. Meta-analysis carried out by Khanakhan with colleagues [13], gives an opportunity to confirm information about global occurrence of OTA in coffee and coffee beverages in different countries. The lowest occurrence of this toxin was in coffee from the South Korea (3 %), Vietnam (10 %) and Panama (19 %); on the contrary, all samples from Kuwait and Chile were contaminated [13]. OTA was detected not only in green coffee [10, 14, 16] but also in their processing products [8, 11, 12, 17–19]. Coffee contamination with AFL and sterigmatocystin (STC) is reported less frequently [5, 10, 20, 21]. Garcia-Moralez and others found out that all natural coffee samples were contaminated with AFL B1 at the range from 0.25 to 2.33 μg/kg [22]; according to Bessiare with colleagues, 18 % of green coffee samples contained from 0.1 to 1.2 μg/kg AFL B1 [10].

The development and implementation of precise analytical multi-detection methods has allowed carrying out a comprehensive assessment of the contamination of coffee and coffee beverages with a large number of MTs, as well as emergent MTs (EMTs). Fumonisins B2 and B4 have been detected in coffee beans [23]. Other types of coffee and beverages were contaminated with enniatins (ENNs) B, B1, A1, alternariotoxin (alternariol monomethyl ether, AME), beauvericin (BEA), citrinin (CIT) and patulin, fumonisin B1 (FB1), trichothecene MTs and mycophenolic acid (MPA) [8, 10, 17–19, 24]. It has been established that coffee roasting may reduce OTA contents by 97 % depending on the temperature and the particle size [25].

Several countries have established hygienic regulations for MTs contents in coffee. Thus, in the RF the maximum level (ML) of AFL B1 should not exceed 0.005 mg/kg[1]; in the European Union ML OTA is 5 μg/kg in roasted coffee beans and ground roasted coffee and 10 μg/kg in soluble coffee (instant coffee) [26]. OTA contents in green coffee are also regulated in Italy, Finland and Greece at the MLs equal to 8, 10 and 20 μg/kg accordingly [5].

Up to now, an issue related to chicory contamination with MTs in the world has scarcely been given any attention. In the RF, any data on MT contamination of coffee and chicory consumed in the country are practically unavailable.

Our research goal was to evaluate frequency and levels of a wide range of toxicological metabolites: MTs regulated in plant food products (AFL B1, B2, G1, G2; OTA, DON, FB1, FB2, T-2, ZEN); their derivatives

[1] TR TS 021/2011. O bezopasnosti pishchevoi produktsii: tekhnicheskii reglament Tamozhennogo soyuza (s izmeneniyami na 14 iyulya 2021 goda) / utv. resheniem Komissii Tamozhennogo soyuza ot 09.12.2011 № 880 [CU TR 021/2011. On food products safety: the technical regulations of the Customs Union (last amended on the July 14, 2021), approved by the Decision of the Customs Union Commission on December 09, 2011 No. 880]. *KODEKS: electronic fund for legal and reference documentation*. Available at: https://docs.cntd.ru/document/902320560 (November 11, 2021) (in Russian).

and structural analogues (DAS, HT-2, T-2 triol, NEOS - derivative of T-2 toxin; 3- and 15-acDON, FUS X – DON derivatives); cyclopiazonic acid (CPA); citreoviredin (CTV); CIT; as well as EMTs (STC, MPA, ENN A and ENN B, beauvericin (BEA), tentoxin (TEN); toxins produced by *Alternaria* sp. such as alternariol (AON), AME and altenuene (ALT)) in the different coffee varieties including green and black coffee (ground coffee and coffee beans) and chicory.

Materials and methods. Coffee and chicory samples were purchased in retail outlets of Moscow and the Moscow region. Overall, we analyzed twenty samples of green coffee beans (Arabica and Robusta varieties from Central and South America, Africa, India and Indochina) and twelve roasted coffee samples, both beans (7 Arabica samples) and ground coffee (5 samples). Chicory samples included 15 instant ones (13 powder-like and 2 liquid) and one sample of roasted chicory radix cut into small pieces.

Prior to extraction, all samples were mixed thoroughly. 50 g portions of coffee beans and roasted chicory radix samples were milled to a homogenous powder. The samples were prepared according to a modified procedure for determining MTs in green coffee suggested by Bessaire et al. [10]. 1.0 g of powder was weighted into 50 ml centrifuge tube, 10 ml of distilled water were added, mixed and allowed to stay for 10 min for swelling. Next, 10 ml of acetonitrile acidified with acetic acid (1 % of volume) were poured into the sample, mixed, ultrasonicated for 10 minutes and shaken for 10 minutes. After addition of 1 g of sodium chloride and 4 g of anhydrous magnesium sulfate the mixture was centrifuged at 4500 rpm for 15 minutes. An extract aliquot (5 ml) was transferred into the 15 ml centrifuge tube and defatted with 3 ml of hexane by shaking for 10 minutes. Then, 0.7 g of anhydrous $MgSO_4$ was added into the tube and stirred. 3 ml portion of acetonitrile fraction was evaporated to dryness under nitrogen and reconstituted with 100 µl of methanol, followed by 400 µl of milliQ water. After centrifugation, the supernatant was transferred into the chromatography vial for analysis.

Soluble chicory samples were prepared as follows: 1.0 g of thoroughly mixed sample was weighted into 50 ml centrifuge tube, 10 ml of distilled water and 10 ml of acidified acetonitrile (acetic acid, 1 vol. %) were consecutively added and the mixture was thoroughly stirred each time, then it was placed into an ultrasonic bath for 10 minutes and a shaker for 10 minutes. After that, 2 g of sodium chloride were added into the tube and the mixture was centrifuged at 4500 rpm for 15 minutes. 800 µl of the extract were transferred into a microcentrifuge Eppendorf tube and 800 µl of milliQ water were added. After centrifugation, the supernatant was transferred into the chromatography vial for analysis. Each sample was analyzed in two replicates.

Analysis was performed using Vanquish UHPLC system connected with triple quadrupole mass-spectrometric detector with a heated electrospray source TSQ Endura controlled by Xcalibur 4.0 QF2 Software (Thermo Scientific, USA). Analytes were separated on a column filled with octadecyl silica (Titan C18, 2.1 × 100 mm, 1.9 µm, Supelco). The column compartment temperature was 25 °C, the mobile phase flow was 0.4 ml/min, the injection volume was 10 µl.

To perform analysis in positive polarity, the following mobile phases were used: (A) water / methanol (90 / 10 % vol.); (B) methanol / water / acetonitrile (10 / 10 / 80 % vol.), both phases were modified with formic acid (0.1 % vol.) and 1 mM of ammonium formate.

To perform analysis in negative polarity, pH of the phase A was adjusted to 9.0 with aqueous ammonia and equal amount of ammonia was added to the phase B. The gradient scheme was as follows: the start at 0 % B; increase to 100 % B within 20 min; 20–23.5th min, 100 % B; 23.5–24th min, 0 % B; column equilibration at 0 % B from 24th to 26th min.

MS / MS detection was performed in positive or negative electrospray ionization polarities (Tables 1 and 2).

Table 1

MRMs for MTs detected in positive polarity

MT	t_R, min	Parent ion	Parent ion (m/z)	Product ions* (m/z)	Collision energy, V	Fragmentor, V
DON	11.2	$[M+H]^+$	297.1	249.1; 267.1	10.6; 17.9	100
T-2 triol	11.3	$[M+NH_4]^+$	400.2	365.2; 145.2	10; 25	76
FUS X	11.4	$[M+H]^+$	355.4	247.0; 229.1	12.3; 16.0	103
NEOS	11.4	$[M+NH_4]^+$	400.2	215.1; 197.2	16.6; 16.7	79
HT-2	12.3	$[M+NH_4]^+$	442.3	215.1; 263.1	10; 10	91
3- and 15- acDON	13.5	$[M+H]^+$	339.1	137.1; 231.1	10; 12.9	97
AFL G2	14.1	$[M+H]^+$	331.1	245.1; 189.1; 285.1	30; 41; 27	170
AFL G1	14.3	$[M+H]^+$	329.1	243; 200	26; 41	150
FB1	14.4	$[M+H]^+$	722.5	704.5; 352.4	28; 36	217
AFL B2	14.8	$[M+H]^+$	315.1	287.1; 259.0	32; 29	170
DAS	14.9	$[M+NH_4]^+$	384.2	307.2; 247.1	10.3; 14	89
AFL B1	16.1	$[M+H]^+$	313.1	241.0; 213.0	37; 45	166
TEN	16.2	$[M+H]^+$	415.3	312.2; 256.2	19; 29	129
FB2	16.9	$[M+H]^+$	706.5	336.4; 354.4	36; 34	150
MPA	17.5	$[M+H]^+$	321.0	207.0; 303.1	22; 10	113
T-2	18.9	$[M+NH_4]^+$	484.3	215.1; 185.1; 305.2	17; 21; 13	138
CTV	19.1	$[M+H]^+$	403.2	297; 315	10; 10	45
OTA	19.3	$[M+H]^+$	404.1	239; 221	24; 35	123
STC	21.1	$[M+H]^+$	325.1	281.0; 253.0	36; 44	152
CPA	21.8	$[M+H]^+$	337.1	182.0; 196.1	19; 23	165
ENN B	24.6	$[M+NH_4]^+$	657.6	214.2; 527.4	31; 27	142
BEA	25.2	$[M+NH_4]^+$	801.4	244.2; 134.2	32; 54	215
ENN A	25.5	$[M+H]^+$	682.7	210.2; 228.2	24; 24	255

Note: * the first indicated ion is the one used for quantification.

Table 2

MRMs of MTs detected in negative polarity

MT	t_R., min	Parent ion	Parent ion (m/z)	Product ions* (m/z)	Collision energy, V	Fragmentor, V
CIT_1	12.0	$[M+CH_3OH-H]^-$	281	249	10	50
CIT_2	12.0	$[M-H]^-$	249.2	115.2; 205.1	52; 18	200
ALT	14.2	$[M-H]^-$	291.2	189.2; 203	32; 32	188
AON	16.2	$[M-H]^-$	256.9	213; 215; 212.1	22; 25; 32	195
ZEN	20.0	$[M-H]^-$	317.2	175; 73.1; 131.1	23; 18; 28	228
AME	20.1	$[M-H]^-$	271.1	256; 228; 227.1	21; 29; 37	194

Note: * the first indicated ion is the one used for quantification.

The source had the following parameters: the vaporizer temperature was set at 225 °C; spray voltage, 4500 V; the ion transfer tube temperature, 200 °C; sheath gas, 35, aux gas, 10, sweep gas, 2 arb. units (nitrogen in all cases); CID gas (argon), 2 mTorr; dwell time, 100 ms; Q1 and Q3 resolution, 0.7 and 1.4 FWHM accordingly.

Standard solutions of 29 MTs were prepared from dry standards (Sigma Aldrich; Fermentek, Jerusalem, Israel). Stock standards were prepared in acetonitrile (AFL, STC, CIT, trichothecenes of groups A and B, ZEN and its analogues, OTA), methanol (*Alternaria* toxins, ENN A, ENN B, BEA, MPA) or in an "acetonitrile / water" mixture (50 / 50 % vol.)

(FB1, FB2 with a concentration equal to 100 or 500 μg/ml). Standard solutions were used to make a multi-standard and calibration solutions. All the solutions were stored at -18 °C.

To quantify MTs, external calibrations on a "clean" matrix were applied. LOD and LOQ were estimated according to 3-σ and 10-σ criteria: 17 and 52 μg/kg for DON and 3- and 15-acDON toxins; 8.7 and 26 μg/kg for FUS X; 5 and 17 μg/kg for MPA; 4 and 12 μg/kg for T-2 triol; 2.8 and 8.5 μg/kg for CTV and CPA; 2.3 and 6.8 μg/kg for ALT, AON, AME and HT-2 toxin; 1.1 and 3.4 μg/kg for FB1, AFL G2, ZEN and DAS; 0.7 and 2.0 μg/kg for ENN A; 0.5 and 1.7 μg/kg for NEOS, CIT, TEN, ENN B, BEA and T-2; 0.2 and 0.7 μg/kg for OTA and FB2; 0.09 and 0.3 μg/kg for AFL G1 and B2; 0.05 and 0.15 μg/kg for STC and AFL B1. Recovery varied from 60 to 108 %. "Positive" samples were divided into two sub-groups. The first one included samples, containing MTs at concentrations exceeding limit of detection (LOD). The second sub-group included samples contaminated with MT over limit of quantification (LOQ).

Results and discussion. ***Coffee contamination with mycotoxins.*** Occurrence of MTs in 32 samples of green and roasted **coffee** was examined. Six out of twenty-nine analyzed MTs were detected in the samples including MPA, OTA, BEA, AFL B1, AFL B2 and STC. Six percent of green coffee samples were contaminated with regulated AFLs at the levels below 5 μg/kg (0.37 and 1.07 μg/kg). MPA was detected, in 44 % of samples; other toxins were detected less frequently: BEA, in 3 % of the cases; MTs produced by "storage fungi" such as OTA (6 %), AFL B2 (6 %), AFL B1 (3 %). STC was detected in trace quantities (Table 3).

MPA concentrations varied within a wide range from 23.5 to 712.2 μg/kg. All the analyzed samples met the requirements for AFL B1 contents in coffee established in the CU TR 021/2011[1].

A more detailed study of MT contamination of coffee depending on the method of its treatment is presented in Table 4.

The analysis conducted in this study for the first time confirmed the presence of significant amounts of EMTs (MPA and BEA) in the analyzed coffee samples. MPA was detected in 55 % of the green coffee samples (contamination range from 23.5 to 58.3 μg/kg). The occurrence of MPA in roasted coffee was 2 times lower than in ground black and green coffee, while contamination levels amounted to 151.7 and 712.2 μg/kg. BEA was also detected in black ground coffee samples at 0.5 μg/kg.

OTA was detected in isolated samples of roasted coffee beans and green coffee in quantities that were by several times lower than the hygienic standards fixed in the European Union countries. OTA contents in green coffee were by almost 3 times higher than in roasted coffee and amounted to 1.07 μg/kg, which agreed with the published data [5, 10].

Table 3

Occurrence of MTs in coffee and chicory samples

MTs	Samples contaminated with MTs over LOD, %		Contamination, μg/kg	Samples contaminated with MTs over LOQ, %	Contamination levels, μg/kg
	< LOQ	> LOQ			
	Coffee (n = 32)			***Chicory (n = 16)***	
AFL B1	3	–	0.05	6	5.76
AFL B2	6	–	0.09; 0.11	–	–
OTA	3	3	0.37; 1.07	6	1.6
STC	3	–		–	–
MPA	–	44	23.5–712.2	–	–
FB2	6	3	0.2–2.6	–	–
ENN B	–	–	–	38	2.8–1109.0
BEA	3	–	0.50	56	2.4–1173.0

N o t e : *LOD is limit of detection; LOQ is limit of quantification.

Table 4

Occurrence of MTs in green and black coffee

Toxin	A number of samples		MT contents in contaminated samples, μg/kg		MT contents in samples of the whole series, μg/kg		
	analyzed	contaminated	range	average	M	Me	90 %
Green coffee							
MPA	20	11 (55 %)	23.5–58.3	36.6	20.1	24.1	51.3
AFL B2		2 (10 %)	0.09; 0.11	0.10	0.010	0	0.05
OTA		1 (5 %)	1.07	1.07	0.05	0	0
AFL B1		1 (5 %)	0.05	0.05	0.003	0	0
FB2		1 (5 %)	2.60	2.60	0.13	0	0
Roasted coffee beans							
MPA	7	2 (25.6 %)	155.7; 712.2	434.0	124.0	0	155.7
OTA		1 (14.3 %)	0.37	0.1	0.01	0	0.05
Black ground coffee							
MPA	5	1 (20.0 %)	72.8	72.8	14.6	0	36.4
BEA		1 (20.0 %)	0.46	0.46	0.09	0	0.23

Table 5

Chicory contamination with mycotoxins

Toxin	A number of samples		Contamination range, μg/kg	Average contents in contaminated samples, μg/kg	MT contents in samples of the whole series, μg/kg		
	analyzed	contaminated			M	Me	90 %
BEA	16	9 (56 %)	2.4–1176.2	152.8	85.9	2.5	84.5
ENN B		6 (38 %)	2.8–1109.0	390.5	156.2	0	604.8
AFL B1		1 (6 %)	5.76	5.76	0.64	0	0
OTA		1 (6 %)	1.6	1.6	0.1	0	0

We should note that only green coffee samples were contaminated with AFL: in one samples AFL B1 content was 0.05 μg/kg, in two other samples AFL B2 was found in amounts of 0.09 and 0.11 μg/kg. AFL B1, FB2 and trace quantities of STC were detected only in a Robusta coffee sample. Similar results were obtained on the ground coffee samples; trace quantities of OTA were detected only in a mixture of coffee varieties. The higher MT contamination of Robusta coffee compared to Arabica coffee was also reported by Bessaire and others [5].

Several MTs were rarely identified in the same sample. Two samples were simultaneously contaminated with two toxins, MPA+OTA and MPA+AFL B2. Similar results for green coffee were obtained by other researches [10]. The risk assessment of MT contamination of coffee, based on analyzing the results on this sample of coffee of different varieties and methods of preparation, shows that coffee, in comparison with other products of plant origin, is not a significant source of MT. This assessment indicates that coffee cannot be considered a substantial source of MTs in comparison with other vegetative food products.

In this research, MT contamination of **instant chicory** was studied for the first time. Four toxins were detected as contaminants of this product, the emergent fusariotoxins BEA and ENN B being detected most frequently (Table 5).

More than a half of the samples contained BEA in concentrations from 2.4 to 1176.2 μg/kg. Average contents amounted to 152.8 μg/kg in contaminated samples and 85.9 μg/kg in all the analyzed samples. ENN B was detected in six chicory samples in concentrations ranging from 2.8 to 1109.0 μg/kg. Its average contents reached 390.5 μg/kg in contaminated samples; 156.2 μg/kg, in all the series of samples; 604.8 μg/kg, in 90 %.

Attention should be drawn to the detection in single cases of OTA (1.6 μg/kg) and AFL B1 (5.76 μg/kg) which exceeds hygienic regulation of AFL B1 content, established for some types of plant products in the RF (tea, coffee, cacao and cacao-based products, grains and products made of processed grains).

Liquid chicory samples were also contaminated with EMTs. One sample contained 2.8 μg/kg of ENN B and 3.6 μg/kg of BEA; the other, 1060.2 μg/kg of ENN B and 1172.6 μg/kg of BEA. We should note that AFL B1 that is regulated in food products of plant origin was detected in roasted chicory in the amount of 5.76 μg/kg, together with BEA (3.0 μg/kg).

Conclusions:

1. The procedure for quantification of mycotoxins by ultra-high performance liquid chromatography coupled to tandem mass-spectrometric detection in coffee and chicory has been developed. We have optimized conditions for chromatographic separation and mass-spectrometric detection of 29 MTs. The procedure has been characterized in terms of recovery, limits of detection and quantification.

2. To the best of our knowledge, it has been the first survey of multi-mycotoxin (29 mycotoxins (MTs)) contamination of coffee and chicory present in the RF market. The results indicated wide occurrence of emergent mycotoxins in these products. Occurrence of EMTs, such as MPA and BEA in green and roasted coffee amounted to 47 %; BEA and ENN B were detected in 94 % of chicory samples. MPA concentrations in coffee reached 712.2 μg/kg; maximum ENN B and BEA contents in chicory equaled 1109 and 1173 μg/kg accordingly.

3. Several MTs regulated in food were detected in coffee and chicory samples: OTA, AFL and FB2 – in coffee samples, and AFL B1 and OTA – in chicory. When such carcinogenic toxins as AFL, OTA and fumonisins occur in plant products, this creates a potential health risk when they are consumed with food.

4. The results of our experimental survey on coffee and chicory being contaminated with MTs and EMTs indicate the necessity to perform profound hygienic assessment of plant products that are imported into the RF, especially from regions with tropical and subtropical climate where the environmental conditions are favorable for vegetation of toxicogenic mold fungi from *Aspergillus, Penicillium, Fusarium,* as well as other poorly studied producers of mycotoxins and emergent mycotoxins.

Funding. This research was funded by Russian Science Foundation grant number 18-16-00077-P “Emergent mycotoxins in food of plant origin: development of methods of determination, survey of occurrence, species-specific characterization of toxigenic moulds, creation of regulatory”.

Competing interests. The authors declare no competing interests.

References

1. Toci A.T., Farah A., Pezza H.R., Pezza L. Coffee Adulteration: More than Two Decades of Research. *Crit. Rev. Anal. Chem.*, 2016, vol. 46, no. 2, pp. 83−92. DOI: 10.1080/10408347.2014.966185

2. Ergin E., Tokusoglu O., Vural H. Coffee toxicology, processing of the coffee and liver diseases (is it a miracle of nature?). *Food Process. Preserv.*, 2021, vol. 45, no. 4, pp. e15243. DOI: 10.1111/jfpp.15243

3. Vidy i sorta kofe [Types and varieties of coffee]. Available at: https://bengusta.com.ua/blog/likbez/vidy-i-sorta-kofe/ (21.11.2021) (in Russian).

4. Ludwig I.A., Clifford M.N., Lean M.E.J., Ashihara H., Crozier A. Coffee: biochemistry and potential impact on health. *Food Funct.*, 2014, vol. 5, no. 8, pp. 1695–1717. DOI: 10.1039/c4fo00042k

5. Vierra V., Cunha S., Casal S. Mycotoxins in coffee. *Coffee in Health and Disease Prevention*, 2015, Chapter 25, pp. 225–233. DOI: 10.1016/B978-0-12-409517-5.00025.5

6. Ramirez L.M., Cendoya E., Nichea M.J., Zachetti V.G.L., Chulze, S.N. Impact of toxigenic fungi and mycotoxins in chickpea: a review. *Current Opinion in Food Science*, 2018, vol. 23, pp. 32–37. DOI: 10.1016/j.cofs.2018.05.003

7. Abdel-Hadi A., Magan N. Influence of physiological factors on growth, sporulation and ochratoxin A/B production of the new *Aspergillus ochraceus* grouping. *World Mycotoxin J.*, 2009, vol. 2, no. 4, pp. 429–434. DOI: 10.3920/WMJ2009.1156

8. Benites A.J., Fernandes M., Boleto A.R., Azevedo S., Silva S., Leitao A.L. Occurrence of ochratoxin A in roasted coffee samples commercialized in Portugal. *Food Control,* 2017, vol. 73, part B, pp. 1223–1228. DOI: 10.1016/j.foodcont.2016.10.037

9. Perez De Obanos A., Gonzalez-Penas E., Lopez De Cerain A. Influence of roasting and brew preparation on the ochratoxin A content in coffee infusion. *Food Additives and Contaminants*, 2005, vol. 22, no. 5, pp. 463–471. DOI: 10.1080/02652030500090042

10. Bessaire T., Perrin I., Tarres A., Bebius A., Reding F., Theurillat V. Mycotoxins in green coffee: Occurrence and risk assessment. *Food Control,* 2019, vol. 96, pp. 59–67. DOI: 10.1016/j.foodcont.2018.08.033

11. Lindenmeier M., Schieberle P., Rychlik M. Determination of ochratoxin A in food: comparison of a stable isotope dilution assay, liquid chromatography-fluorescence detection and an enzyme-linked immunosorbent assay. *Mycotoxin Res.*, 2011, vol. 27, no. 2, pp. 115–121. DOI: 10.1007/s12550-010-0084-1

12. Lee T.P., Saad B., Khayoon W.S., Salleh B. Molecularly imprinted polymer as sorbent in microsolid phase extraction of ochratoxin A in coffee, grape juice and urine. *Talanta*, 2012, vol. 88, pp. 129–135. DOI: 10.1016/j.talanta.2011.10.021

13. Khaneghah A.M., Fakhri Y., Abdi L., Coppa C.F.S.C., Franco L.T., Fernandes de Oliveira C.A. The concentration and prevalence of ochratoxin A in coffee and coffee-based products: A global systematic review, meta-analysis and meta-regression. *Fungal Biol.*, 2019, vol. 123, no. 8, pp. 611–617. DOI: 10.1016/j.funbio.2019.05.012

14. Culliao A.G.L., Barcelo J.M. Fungal and mycotoxin contamination of coffee beans in Benguet province, Philippines. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.*, 2015, vol. 32, no. 2, pp. 250–260. DOI: 10.1080/19440049.2014.1001796

15. Barcelo J.M., Barcelo R.C. Post-harvest practices linked with ochratoxin A contamination of coffee in three provinces of Cordillera Administrative Region, Philippines. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.*, 2018, vol. 35, no. 2, pp. 328–340. DOI: 10.1080/19440049.2017.1393109

16. Ayelign A., De Saeger S. Mycotoxins in Ethiopia: Current status, implications to food safety and mitigation strategies. *Food Control*, 2020, vol. 113, pp. 107163. DOI: 10.1016/j.foodcont.2020.107163

17. Nielsen K.F., Ngemela A.F., Jensen L.B., de Medeiros L.S., Rasmussen P.H. UHPLC-MS/MS determination of ochratoxin A and fumonisins in coffee using QuEChERS extraction combined with mixed-mode SPE purification. *J. Agric. Food Chem.*, 2015, vol. 63, no. 3, pp. 1029–1034. DOI: 10.1021/jf504254q

18. Casal S., Vieira T., Cruz R., Cunha S.C. Ochratoxin A in commercial soluble coffee and coffee substitutes. *Food Res. Int.*, 2014, vol. 61, pp. 56–60. DOI: 10.1016/j.foodres.2014.04.045

19. García-Moraleja A., Font G., Mañes J., Ferrer E. Analysis of mycotoxins in coffee and risk assessment in Spanish adolescents and adults. *Food Chem. Toxicol.*, 2015, vol. 86, pp. 225–233. DOI: 10.1016/j.fct.2015.10.014

20. Karlovsky P., Suman M., Berthiller F., De Meester J., Eisenbrand G., Perrin I., Oswald I.P., Speijers G. [et al.]. Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*, 2016, vol. 32, no. 4, pp. 179–205.

21. Sedova I.B., Kiseleva M.G., Zakharova L.P., Tutelyan V.A. Toxicological and hygienic characteristics of mycotoxin sterigmatocystin and methods for its determination in food products. *Gigiena i sanitariya*, 2019, vol. 98, no. 1, pp. 105–117. DOI: 10.18821/0016-9900-2019-98-1-105-117

22. García-Moraleja A., Font G., Mañes J., Ferrer E. Simultaneous determination of mycotoxin in commercial coffee. *Food Control*, 2015, vol. 57, pp. 282–292. DOI: 10.1016/j.foodcont.2015.04.031

23. Paterson R.R.M., Lima N., Taniwaki M.H. Coffee, mycotoxins and climate change. *Food Research International*, 2014, vol. 61, pp. 1–15. DOI: 10.1016/j.foodres.2014.03.037

24. Bokhari F.M., Aly M.M. Evolution of traditional means of roasting and mycotoxins contaminated coffee beans in Saudi Arabia. *Advances in Biological Research*, 2009, vol. 3, no. 3–4, pp. 71–78.

25. Oliveira G., da Silva D.M., Pereira R., Paiva L.C., Prado G., Batista L.R. Effect of different roasting levels and particle sizes on ochratoxin A concentration in coffee beans. *Food Control,* 2013, vol. 34, no. 2, pp. 651–656. DOI: 10.1016/j.foodcont.2013.06.014

26. COMMISSION REGULATION (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*, December 20, 2006. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX: 32006R1881&from=EN (01.11.2021).

Sedova I.B., Kiseleva M.G., Chalyy Z.A. Mycotoxins in coffee and chicory: from regulated to emergent. Health Risk Analysis, 2022, no. 2, pp. 64–72. DOI: 10.21668/health.risk/2022.2.06.eng

Received: 30.11.2021
Approved: 13.04.2022
Accepted for publication: 21.06.2022