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Research Article

Determination of aflatoxin and some trace element contents in black pepper samples

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ABSTRACT

Concentrations of the total aflatoxin (AF), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2) and some trace elements such as lead, cobalt, nickel, arsenic, copper, zinc, manganese, magnesium, iron, calcium and chromium in black pepper samples were determined. Four black pepper samples were collected from markets in Karaman/Turkey. Detection of toxins were performed using HPLC instruments after pre-separation using immunoaffinity columns working through a solid phase extraction. The heavy metal contents in black peppers were determined by flame atomic adsorption methods. Also, quantities of trace elements were determined in 4 samples collected during summer and winter months. The total AF was determined below the maximum limits in pepper samples. Quantities of Co, Ni, Cr, Cu, Zn, Pb, Mn, Fe, Mg and Ca were detected in the samples. When the results were evaluated, the values found for Cu, Zn and Mg were seen to be at normal levels. Co, Ni, Cr, Pb, Mn, Fe, Ca trace element concentrations were obtained above the normal limit but Co, Pb and Mn were found below the toxic limits.

Keywords: Aflatoxin, heavy metals, HPLC, FAAS.

Karabiber örneklerinde aflatoksin ve bazı eser element içeriklerinin belirlenmesi

ÖZ

Karabiber örneklerinde toplam aflatoksin (AF), aflatoksin G1 (AFG1), aflatoksin G2 (AFG2), aflatoksin B1 (AFB1), aflatoksin B2 (AFB2) ve kurşun, kobalt, nikel, arsenik, bakır, çinko, manganez, magnezyum, demir, kalsiyum ve krom gibi bazı eser elementlerin konsantrasyonları belirlenmiştir. Dört adet karabiber örneği Karaman/ Türkiye'nin pazarlarından toplanmıştır. Toksinlerin belirlenmesi, bir katı faz ekstraksiyonu ile çalışan immünoafinite kolonları kullanılarak ön ayırma işleminden sonra HPLC cihazı kullanılarak gerçekleştirilmiştir. Karabiberlerdeki ağır metal içerikleri alevli atomik adsorpsiyonmetodu ile belirlenmiştir. Ayrıca, yaz ve kış aylarında toplanan 4 örnekte eser element miktarları belirlenmiştir. Biber örneklerinde toplam AF, maksimum sınırların altında belirlenmiştir. Örneklerdeki Co, Ni, Cr, Cu, Zn, Pb, Mn, Fe, Mg ve Ca miktarları tespit edilmiştir. Sonuçlar değerlendirildiğinde Cu, Zn ve Mg için bulunan değerler normal seviyelerde olduğu görülmüştür. Co, Ni, Cr, Pb, Mn, Fe, Ca eser element konsantrasyonları normal sınırın üzerinde elde edilmiştir, ancak Co, Pb ve Mn toksik sınırların altında bulunmuştur.

Anahtar Kelimeler: Aflatoksin, ağır metaller, HPLC, FAAS.

1. INTRODUCTION

Mycotoxins are types of compound that can be found everywhere by fungal spores known to have harmful influences on human and animal health. Mycotoxins that can occur in cereals, nuts, fruits and green coffee beans endanger food safety.[1] The most widespread mycotoxins are aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin B1

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(AFB1), aflatoxin B2 (AFB2).[2] The presence of mycotoxins causes serious problems in human health.[3] Therefore, the presence of mycotoxins should be eliminated or reduced.[3] The most important factors causing aflatoxin formation in foods are temperature, humidity, drying and storage conditions.[4, 5] Aflatoxins are strongly influenced by ultraviolet light throughout the ring structure. Aflatoxin B1 gives blue fluorescent light (425 nm) under UV light.[6] Since heavy metals are present in the soil, in the air and in the water, they can also be found in all foodstuffs. The amount of metal deposited in food depends on the conditions at which food is produced and processed. Also, it has nutritional value in many metals and is essential for human health. Some elements such as lead, cadmium, mercury, arsenic and thallium have no nutritional value and these elements are taken into the body, causing health problems.[7] The contents of trace elements that can be found in foodstuffs are mostly determined by spectrometric techniques such as ICP-OES or AAS.[8,9] The relationship between heavy metals and mycotoxin compounds that can be found in food products is not mentioned in the literature. In this study, concentrations of the total aflatoxin, aflatoxin G1, aflatoxin G2, aflatoxin B1, aflatoxin B2, lead, cobalt, nickel, arsenic, copper, zinc, manganese, magnesium, iron, calcium and chromium in black pepper samples were determined.

2. MATERIALS AND METHODS

4 samples were analysed for AFs and trace element contents. The samples were obtained from Karaman province in Turkey. Samples were collected winter and summer seasons and checked for AFs levels. At least 100 g of the sample was collected and transported to the laboratory in containers that do not cause pollution.

2.1. Sample preparation for aflatoxins analyses

All samples were ground with a mixer to obtain the appropriate particle size. It was stored in a glass container in a refrigerator until analysis. The AOAC Official Method 999.31 was used to detect AFs in black pepper samples.[10] In this method, extraction with methanol-water mixture, cleaning with IAC and post-column derivatization with kobra cell were performed. The aflatoxins were detected in the liquid chromatography device coupled with the fluorescence detector. 125 g of methanol-water mixture (70:30, v/v) and 5 g of NaCl were added 25 g of finely ground sample. The mixture was stirred at high speed for 2 minutes using a Waring stirrer. The mixture was filtered through filter paper. 15 ml of the resulting filtrate was diluted with 30 ml of water, shaken well and filtered through a microfiber filter (Wicam). It was then passed through an AflaPrep column, which was attached to a vacuum manifold at a flow rate of about 1 ml per minute, taking from a 15 ml diluted filtrate. The column was washed twice in succession with 10 ml of ultrapure water. AFs bound to the certain antibody were eluted by passing 1ml of methanol through the column and collected in HPLC vials. The solution was stored at 2-8°C until HPLC analysis and diluted with 1 ml of ultrapure water just before analysis.

2.2. Sample preparation for heavy metals analyses

All samples were prepared for the analysis using the wet burning method to be parallel to 2 samples each sample. For this purpose, one gram of washed, dried and ground-sized powder sample is weighed. 16 ml of HNO_3 (65%, w/w) and 4 ml of $HClO_4$ (70-72%, w/w) were added. The solution is slowly heated on the heater for about 5-6 hours. The heating process close to the last of the acids was cut off and the solutions were cooled. Then 5 ml of H_2O_2 (30%, w/w) was added the solution and heating was continued until a clear liquid was obtained. After the heating was released, the solutions were allowed to cool. The cooled mixtures were filtered through blue band filter paper and mixed with purified water to make 30 ml solution for analysis. The concentrations of the selected elements were determined by Flame Atomic Absorption Spectrometry. Quantity and field data of aflatoxin standards and calibration curve of aflatoxin B1 standard are given Table 1 and Figure 1, respectively. HPLC-FLD chromatograms of aflatoxin standards and chromatographic parameters used in HPLC analysis are given Figure 2 and Table 2, respectively.

2.3. Chromatographic separations

The fluorescence detector was set to an excitation and emission wavelengths of 360 and 430 nm, respectively. The retention times were around 7.245, 8.427, 9.414 and 11.57 min for AFG2, AFG1, AFB2 and AFB1, respectively. Linearity, recovery, accuracy, and sensitivity were detected to appraise the performance of analytic method used for aflatxins (AFs). To define linearity, five-point calibration graphs were drawn over the concentration range of 0.1-2 µg l^{-1} for each AF. Linear lines were plotted using the peak area against the standard concentration. The linearity was defined by linear regression analysis and expressed as coefficient of determination (R^2). The detection limits of analytical methods (LOD) and quantitative limits (LOQ) were determined according to the EURACHEM Guide recovery test.[11] Blank black pepper samples were spiked with 0.1 mg kg^{-1} for each analyte and measured in 6 independent replicates. LODs and LOQs were calculated according to some equations. These equations: LOD = X + 3s, LOQ = X + 10s in which, “X” is the mean concentration of fortified sample blank values, and “s” is the sample standard deflection.

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Table 1. Quantity and field data of aflatoxin standards

	Ref. Amount	RT	Signal	Compound	Lvl	Amt(µg kg^{-1})	Area	ISTD
1	2.5000e-2	7.379	FLD1A	Aflatoxin G2	1	2.5000e-2	8.0166e-1	No
	6.2500e-2				2	6.2500e-2	2.027	
	1.2500e-1				3	1.2500e-1	4.007	
	0.250				4	0.250	8.084	
	0.500				5	0.500	16.225	
2	0.100	8.652	FLD1A	Aflatoxin G1	1	0.100	2.643	No
	0.250				6	0.250	6.534	
	0.500				7	0.500	13.042	
	1.000				8	1.000	25.965	
	2.000				9	2.00	51.656	
3	2.5000e-2	9.663	FLD1A	Aflatoxin B2	1	2.5000e-2	1.697	No
	6.2500e-2				2	6.2500e-2	4.15	
	1.2500e-1				3	1.2500e-1	8.129	
	0.250				4	0.250	16.47	
	0.500				5	0.500	32.679	
4	0.100	11.532	FLD1A	Aflatoxin B1	1	0.100	3.29	No
	0.250				6	0.250	7.735	
	0.500				7	0.500	15.326	
	1.000				8	1.000	30.405	
	2.000				9	2.00	60.234	

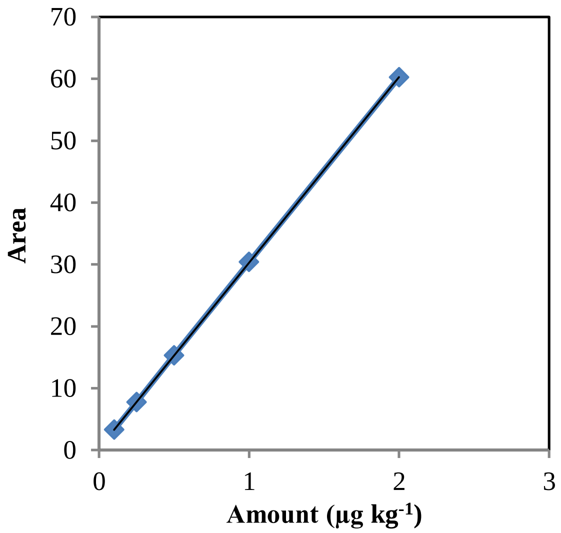

Figure 1. Calibration curve of aflatoxin B1 standard.

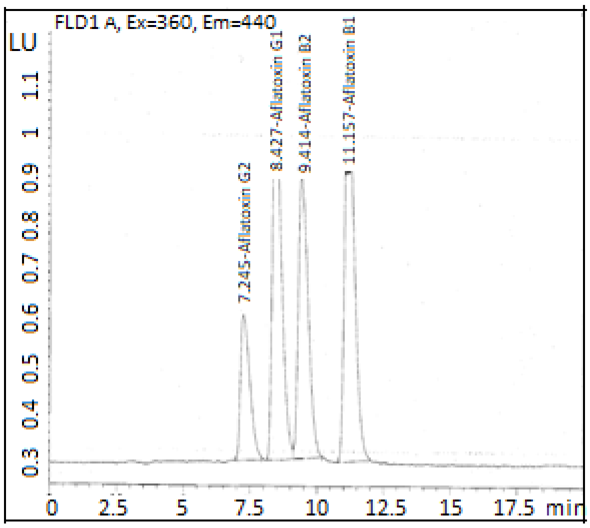

Figure 2. HPLC-FLD chromatograms of aflatoxinstandards.

Linearity data and method performance characteristics for aflatoxins and standard mixing solution concentrations which have been prepared for calibration curves are given Tables 3 and 4.

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Table 2. Chromatographic parameters used in HPLC analysis

	Method
Column temperature	33
Column flow (ml dk^{-1})	1.0
Injection volume (µl)	20
Detection wavelength (nm)	202, 212, 219, 222, 263, 265, 279
Mobile phase A	Water/Acetonitrile/Methanol+KBr+HNO_3
Column	C18 (250 × 4.6mm, 5µm)
Dedector	Fluorescence (ex: 360 nm, em: 435)

Table 3. Linearity data and method performance characteristics for aflatoxins

Aflatoxins	Linearity Range (µg kg^{-1})	Lineer Regression Equation	R²	LOD (µg kg^{-1})	LOQ (µg kg^{-1})
AFB1	0.1-2	y = 4.67x + 0,17	0.9999	0.11	0.13
AFB2	0.1-2	y = 6.86x + 0,11	0.9997	0.11	0.13
AFG1	0.1-2	y = 3.89x + 0,14	0.9998	0.12	0.14
AFG2	0.1-2	y = 2.96x + 0,12	0.9996	0.12	0.14

R^2: Coefficient of determination. LOD: Detection limit of the chromatographic method. LOQ: Quantification limit of the chromatographic method.

Table 4. Standard mixing solution concentrations which have been prepared for calibration graphics (mg kg^{-1})

ELEMENT	STANDART NO								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Ca	1.0	2.0	4.0	8.0	12.0	16,0	32.0	64.0	72.0
Pb	1.0	2.0	4.0	6.0	12.0				
Co	1.0	2.0	3.0	4.0	5.0	6.0	12.0		
Cr	1.0	2.0	3.0	4.0	5.0	6.0			
Cu	1.0	2.0	3.0	4.0	5.0	6.0			
Fe	1.0	3.0	5.0	7.0	9.0	18.0	36.0	54.0	72.0
Mg	0.5	1.0	2.0	4.0	6.0	12.0	24.0		
Mn	1.0	2.0	3.0	4.0	5.0	10.0	20.0		
Ni	1.0	2.0	3.0	4.0	5.0	6.0			
Zn	1.0	2.0	3.0	4.0	5.0	10.0	20.0	40.0	

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3. RESULTS AND DISCUSSION

Aflatoxin concentrations in black pepper collected in winter and summer are given in Tables 5 and 6.

Table 5. Aflatoxin concentrations in black pepper collected in winter (μg kg^{-1})

Sample	B1	B2	G1	G2
1	0.012	-	0.157	-
2	0.011	0.009	0.145	-
3	0.013	0.008	0.143	0.016
4	0.012	-	-	0.014
Average ± SD	0.012 ± 0.0008	0.085 ± 0.007	0.148 ± 0.007	0.0015 ± 0.001

Table 6. Aflatoxin concentrations in black pepper collected in summer (μg kg^{-1})

Sample	B1	B2	G1	G2
1	-	-	0.043	0.015
2	-	-	0.042	0.016
3	-	-	0.045	0.014
4	0.003	-	0.048	0.013
Average ± SD	0.003	-	0.044 ± 0.002	0.014 ± 0.001

Aflatoxin levels in black pepper collected in winter and summer, and aflatoxin and total aflatoxin levels in samples are given in Figures 3-5. The average concentrations of trace elements in the black peppers are given in Table 7. Average trace element concentrations in black pepper samples are given in Figures 6-8. Average element contents and assessment in foodstuffs are given in Table 8.

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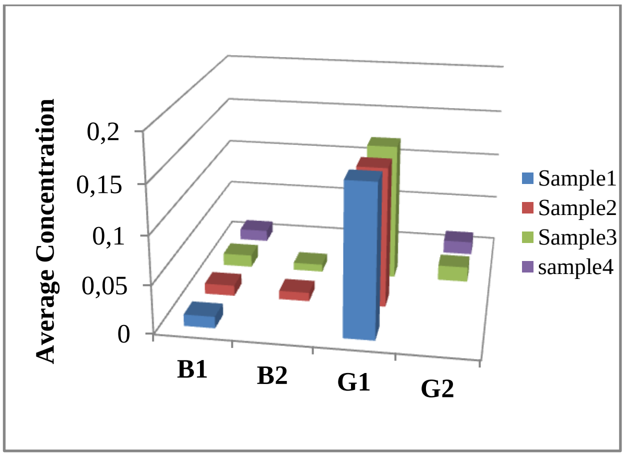

Figure 3. Aflatoxin levels in black pepper collected in winter (µg kg^{-1}).

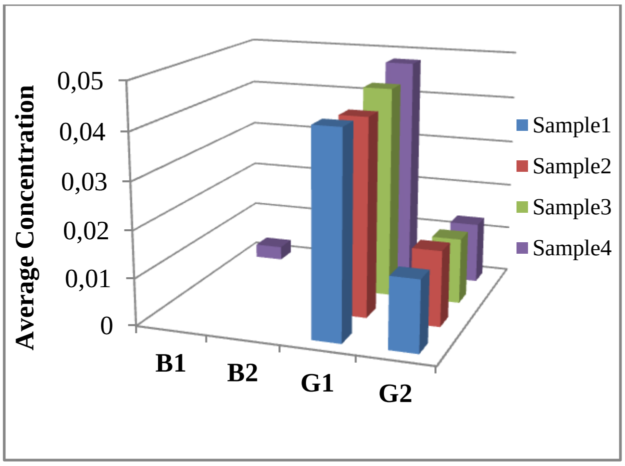

Figure 4. Aflatoxin levels in black pepper collected in summer (µg kg^{-1}).

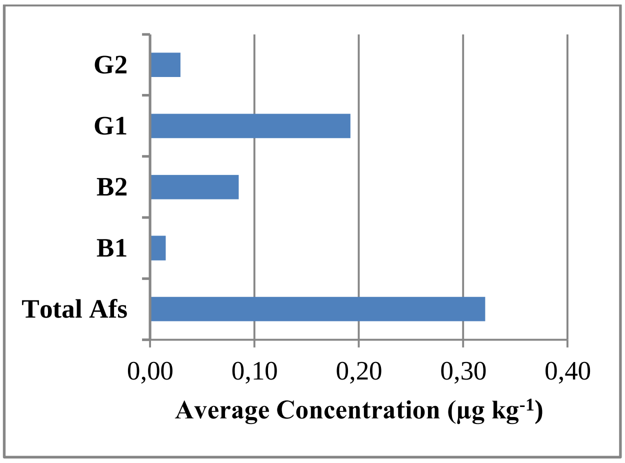

Figure 5. Aflatoxin levels and total aflatoxin in samples (µg kg^{-1}).

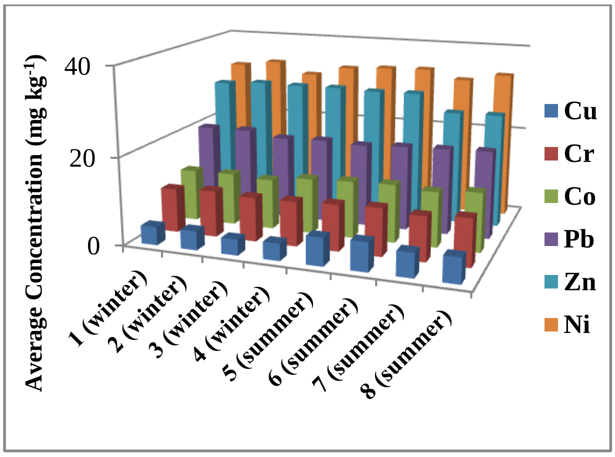

Figure 6. Average trace element (Cu, Cr, Co, Pb, Zn, Ni) concentrations in black pepper samples (mg kg^{-1}).

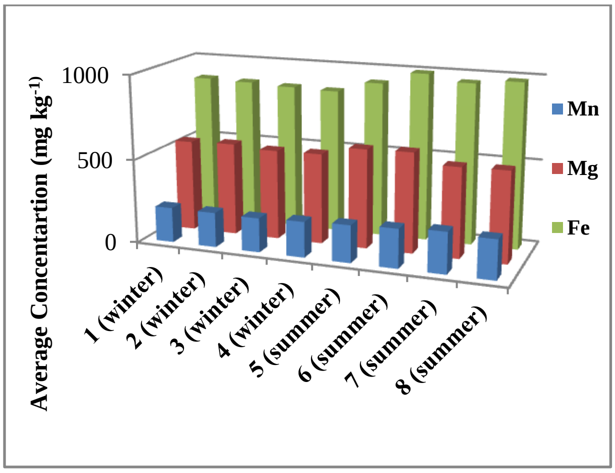

Figure 7. Average trace element (Mn, Mg, Fe) concentrations in black pepper samples (mg kg^{-1}).

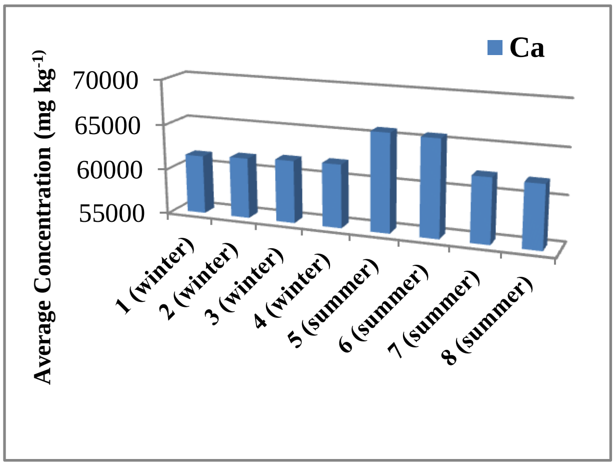

Figure 8. Average trace element (Ca) concentrations in black pepper samples (mg kg^{-1}).

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Table 7. The average trace element concentrations in the black peppers (mg kg^{-1})

Black Pepper	Co	Ni	Cr	Cu	Zn	Pb	Mn	Mg	Fe	Ca
Winter	11.81	32.41	10.03	4.17	29.34	20.09	208.3	544	882	61480
	11.96	33.61	10.55	4.32	29.97	20.19	206.7	552	875	61690
	11.47	31.03	10.04	3.60	29.87	19.01	203.5	535	864	61910
	12.62	33.16	10.21	3.90	29.99	19.31	211.9	539	857	62010
Summer	13.03	33.72	10.57	6.37	29.73	18.96	222.1	591	922	65860
	13.27	33.99	10.88	6.55	29.91	19.48	232.1	597	996	65710
	12.62	32.02	10.16	5.49	25.99	19.77	245.3	537	958	62160
	13.44	33.68	10.85	5.73	26.14	20.07	234.2	542	983	62000
Average ±SD	12.53 ± 0.718	32.95 ± 1.037	10.41 ± 0.347	5.016 ±1.157	28.86 ± 1.742	19.61 ± 0.491	220.5 ± 15.32	554.6 ± 24.89	917.1 ± 55.67	62852 ± 1822

Table 8. Average element contents and evaluation in foodstuffs (mg kg^{-1})[9,12]

Element	Range Found	Normal Concentration	Toxic Concentration	Evaluation
Co	11.47 – 13.44	0.05 -0.50	30 - 40	High
Ni	31.03 - 33.99	0.10 -5.00	30.0	High
Cr	10.03 – 10.88	0.10 -1.00	2.00	High
Cu	3.60 - 6.55	3.00-15.0	20.0	Normal
Zn	25.99 – 29.99	15.0 - 150	200	Normal
Pb	18.96 – 20.19	1.00- 5.00	20.0	High
Mn	203.5 – 245.3	15.0 - 100	400	High
Fe	857 – 996	50.0 - 250	500	High
Mg	535– 597	250 - 3500	--	Normal
Ca	61480 - 65860	90 - 12000	--	High

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When the results were evaluated, Cu, Zn and Mg were found to be normal in the determined limits. Co, Ni, Cr, Pb, Mn, Fe, Ca element concentrations were found above the normal range, but Co, Pb and Mn below the toxic limits. Europen quantification limits were 0.11, 0.11, 0.12 and 0.14 µg kg^{-1} for AFB1, AFB2, AF G1 and AFG2, respectively. AFs are present in 100% of black pepper at total AFs levels ranging. Four black pepper samples were found below the European maximum tolerable limit (MTL) for AFB1 and total AFs concentration.

4. CONCLUSIONS

In this study, concentrations of the total aflatoxin, AFG1, AFG2, AFB1, AFB2, lead, cobalt, nickel, arsenic, copper, zinc, manganese, magnesium, iron, calcium and chromium in black pepper samples were determined. Minerals should be taken less than 100 mg per day. These elements make up less than 0.01% of body weight. The basic trace elements required for human health are silicon, zinc, manganese, iron, copper, chromium, fluoride and iodine. Basic minerals function as structural components of textures.[10] In addition, these elements are active in cellular and basal metabolism, water and acid-base balance in the human body.[10] Prevention of heavy metal contamination, sources of contamination should be identified. These resources should be eliminated or reduced. Inspections of potentially dangerous sources should be carried out continuously. Codex Alimentarius has developed various application codes to prevent and reduce the formation of AFs in foods. Producers should produce food according to the rules, taking into account the Good Agricultural Practices (GAP). Good Storage Practices (GMP) and Good Food Production (GMP) rules should be applied during the processing and storage of food after production.[13]

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Conflıct of ınterest

Authors declare that there is no a conflict of interest with any person, institute, company, etc.

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