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RESEARCH ARTICLE

The effects of aflatoxin residues on nutritional contents in ground red chili peppers (*Capsicum annuum*)

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ABSTRACT

We aimed to determine and compare the nutrient and total aflatoxin (AF) content of ground red chili peppers from three different highest production regions of Turkey. Of the collected samples, 51.12% were found to be contaminated with AFs, while only 28.89% contained AF over maximum residue limits. AFB1 contamination in peppers was directly correlated to the amount of carbohydrate, while an opposite correlation was recorded for fat, crude fiber, metabolic energy, radical scavenging activity, total phenol, and ascorbic acid content. Moisture, ash, capsaicinoids, total carotene, and element contents did not differ significantly in AF-contaminated samples.

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Aflatoxins; nutrient; ground red chili pepper; capsaicinoids; ascorbic acid; elements; ascorbic acid

Introduction

Although the *Solanaceae* family of peppers (*Capsicum* sp.) is the homeland of America, it is grown in many parts of the world today, especially in temperate regions (Santos *et al.* 2010). *Capsicum* species are widely used for nutrition and as food flavoring (Zou *et al.* 2015). Numerous studies have shown that they are rich in protein, fat, and minerals and important for maintenance of health in terms of their essential amino acid and fatty acid ingredients (Park *et al.* 2006, Topuz and Ozdemir 2007, Ogunlade *et al.* 2012, Zou *et al.* 2015).

As in other plant origin nutrients, red pepper contents vary considerably, according to planting variety (cultivar) and environmental conditions (Bae *et al.* 2012). For example, it has been reported that *Capsicum* grown in different geographical conditions in USA (Jarret *et al.* 2013), Korea (Park *et al.* 2006), and China (Zou *et al.* 2015) has quite different chemical compositions.

Turkey has a share of less than 1% in the world spicy dry red pepper production; although it is in the second place in the world in the production of fresh peppers (228,531 tons of red peppers were produced in 2016 in 12,241.5 hectares). Peppers encounter problems during export primarily due to aflatoxin (AF) content/contamination (Akbay *et al.* 2012). Although there is significant research in AF content of red peppers grown in Turkey, there is limited research on their nutrient ingredients. Previous studies have analyzed contents of some *Capsicum* species grown in Turkey in terms of their carotenoids, capsaicinoids, ascorbic acid (Poyrazoğlu et al. 2005, Topuz and Ozdemir 2007), and some elements (Karadaş and Kara 2012). Despite the beneficial nutritive value of peppers, AF contamination is a serious global health concern, due to their potent hepatotoxic and carcinogenic effects (Rosas-Contreras *et al.* 2016). It has been shown that they are found in various herbal (Basalan *et al.* 2004) and animal foods (Filazi *et al.* 2010). Many people in Turkey and around the world usually use red chili pepper as a spice to their food. However, consumption of red peppers contaminated with AFs may seriously affect human and animal health and nutrition. Failure to observe hygienic measures during the production, storage, and processing of peppers results in fungal growth and, ultimately, the formation of AFs (Santos *et al.* 2010). For the protection of human health, the maximum residue limit (MRL) in ground red chili peppers in Turkey and European Union countries were determined as 10 μg kg^{-1} for total AFs (B1 + B2 + G1 + G2) and 5 μg kg^{-1} for

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aflatoxin B1 (AFB1) by Commission Regulation EC No. 1881/2006.

AFs not only affect public health, but also affect the nutritional quality of *Capsicum*. For example, it has been reported that *Aspergillus flavus*, produced in ground red peppers, causes losses in total carotenoids (88.55%), total sugars (85.5%) mostly, but increases protein content from 18 to 23% (Tripathi and Mishra 2009). It has also been suggested that the capsaicin (CAP), found only in the *Capsicum*, suppresses the growth of certain bacteria and fungi (Norton 1997, Santos *et al.* 2010). However, all these studies are *in vitro* and experimental. To the best of our knowledge, there are no studies on how fungi, grown in the natural environment, and AFs they secrete affect the nutrient content of flake peppers. Thus, in this study, we aimed to (1) determine some nutrient contents and AF contamination ratio of chili peppers obtained from three different pepper-producing regions of Turkey, (2) compare the nutrients of AF-free chili peppers with AF-contaminated chili pepper, and (3) to investigate the effects of AF contamination on the nutritional ingredients of the peppers by this way.

Materials and methods

Samples

Forty-five dry ground chili pepper samples (15 samples from each) were collected from three most common pepper-producing regions of Turkey (Sanliurfa, Kahramanmaras, and Gaziantep) from different producers. These regions are geographically located in the South Eastern region of Turkey, neighboring each other and constitute more than 70% of domestic red pepper production. Retail market pepper samples of 200 g, were collected in paper bags pursuant to national regulations.

Analysis of AFs in ground red chili pepper

Aflatoxin analysis (AFB1, B2, G1, and G2) were made by the method of Karaaslan and Arslanğray (2015). Briefly, 25 g of the sample was mixed with 5 g of NaCl and homogenized at high speed for 1 min after adding 100 ml of methanol:water (80:20, *v*:*v*). It was filtered through Whatman No. 4 filter paper and to 10 ml extract, 40 ml of deionized water was added, mixed thoroughly. Two drops per second ran 8 ml of the diluted extract through an immunoaffinity column (AflaPrep, R-Biopharm Rhone Ltd., Glasgow, Scotland). The column was washed with 2×10 ml methanol:-water (80:20, *v*:*v*) (flow rate 2 drops/sec). One milliliter of this was diluted with 1 ml of methanol and homogenized. Twenty microliters of this mixture was injected into high performance liquid chromatography (HPLC). The derivatization process was performed with the Kobra Cell^{TM} (Rhone Diagnostics Technologies Ltd., Glasgow, UK) electrochemical bromination system. Identification of AFs was performed with a Thermo Finnigan HPLC instrument using an automatic injector and a fluorescence detector. Zorbax Eclipse XDB C18 column (150 mm $\times$4.6 $\times$ 5 μm) was used. The method was validated.

Analysis of capsaicinoids in ground red chili pepper

Analytical-grade CAP (>99%) and dihydrocapsaicin (DHCAP) (>%97) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Stock solutions of capsaicinoids were prepared by weighing and dissolving each compound in HPLC grade methanol. These solutions were stored at 4 °C and used for the preparation of working diluted standard solutions in methanol. Analyses of capsaicinoids (CAP and DHCAP) from chili peppers were done by modifying the method of Peña-Alvarez *et al.*, (2012). For homogenization, 0.25 g of chili pepper was placed in a 50-ml glass tube with 10 ml methanol and homogenized for at least 5 min. After homogenization, the mixture was placed in an ultrasonic bath at 45 °C for 25 min. The sample was then centrifuged at $3500 \times g$ for 10 min, supernatant filtered through 0.2 μm Millipore filter. From the filtrate, 10 μL was removed and diluted with 900 μL of methanol and 1 μL was injected into gas chromatography-mass spectrometer (GC-MS). All injections were carried out in triplicate. A Polaris Q External Ionization Ion Trap GC-MS was used in combination with a split/splitless injector (Thermo Finnigan, San Joe, CA) (electronic ionization: 70 eV). The injector was equipped with a 12 cm $\times$ 5 mm i.d. Silcoseeve liner (Thermo Finnigan) and was operated in the splitless mode and a 2 μL sample volume. Chromatographic separation was performed using an HP-5MS capillary column (30m $\times$ 0.25 mm i.d., 0.25 μm film thickness) (Agilent Technologies, Palo Alto, CA). The injector, transfer line, and external ion source temperatures were kept at 260, 280, and 230 °C, respectively. The carrier gas was helium (purity 99.995%) at a constant flow rate of 1.0 ml min^{-1}. GC oven program started at 40 °C (hold time 2 min), which was raised at 10 °C min^{-1} to 300 °C and hold 5 min. Mass spectra (*m*/*z* 50–550) were recorded at a rate of five scans per second with an

ionization energy of 70 eV. This method has been validated.

Analysis of total carotenoids in ground red chili pepper

The total carotenoid analysis was performed by spectrophotometric (Shimadzu UV 1601, Tokyo, Japan) method defined by Hwang *et al.* (2012). The carotenoids were extracted repeatedly by homogenizing until 0.5 g of sample became colorless with acetone:-petroleum ether mixture (1:1, *v*:*v*). The extracts were washed twice with deionized water several times in a separatory funnel. The upper phase was filtered through anhydrous sodium sulfate, placed in a 50 ml tube and filled to volume with petroleum ether, homogenized and Absorbance read at 450 nm. From the linear regression curve obtained from the standards, the total carotene amounts of the samples were calculated from the β-carotene.

Analysis of ascorbic acid in ground red chili pepper

Ascorbic acid was identified by HPLC-DAD (Thermo Finnigan) according to the method reported by Topuz and Ozdemir (2007). Briefly, 20 g of the sample was homogenized with 80 ml of 3% meta-phosphoric acid containing 10^{-6} M EDTA and 10^{-7} M diethyldithiocarbamic acid and centrifuged at $5000 \times g$ for 10 min. The upper phase was filtered through a Sep-Pak C18 cartridge (Waters Associates, Milford, MA) pretreated with 3 ml of a 3% meta-phosphoric acid solution and then filtered through a 0.45 μm membrane filter. Chromatographic separation was carried out with reversed phase column (Nucleosil 5 C18, 250 × 4 mm i.d.).

Determination of proximate composition in ground red chili pepper

Moisture, total ash, crude fiber, crude oil, crude protein, and total carbohydrate content were determined by official standard methods and expressed in percentages (AOAC 1998). Moisture was determined by calculating the weight loss of the sample dried for 5 h in an oven heated to 105 °C. The total ash content was measured by incinerating the 2 g sample in a 550 °C oven for 6 h and then weighed the residue after cooling to room temperature in a desiccator. The crude oil content was determined by continuous extraction for 6 h on a Soxhlet device using petroleum ether. The crude protein content was calculated from the nitrogen content using the Kjeldahl method ($N \times 6.25$). Carbohydrate content was determined by subtracting the mean values of the other parameters from 100. Thus, calculated as (carbohydrate %) = 100 − (moisture% + total ash% + crude protein% + crude oil %). Crude fiber content was determined by the addition of soluble and insoluble fractions according to the enzymatic-gravimetric method. Metabolizable energy values were calculated using the formula below (Aremu *et al.* 2011, Ogunlade *et al.* 2012).

$$\text{Metabolizable energy (kJ/kg)} = (\text{Crude protein} \times 17) + (\text{carbohydrate} \times 17) + (\text{crude fat} \times 37)$$

Determination of phenol content in ground red chili pepper

Total phenol content was determined spectrophotometrically using the Folin–Ciocalteu method (Singleton *et al.* 1999). One gram of the sample was homogenized in 25 ml methanol for 15 min in an ultrasonic bath and filtered through Whatman No.1 filter paper. To 0.5 ml of the extract, 2.5 ml of Folin–Ciocalteu reagent and 2 ml of sodium carbonate solution (7.5%, *w*/*v*) were added in glass test tubes. Absorbance of the mixture was measured at 765 nm after 2 h in the dark at room temperature (UV-VIS spectrophotometer, Shimadzu 1601). Gallic acid was used as standard and the total phenol content was calculated as gallic acid equivalent to mg/100 g material.

Determination of radical scavenging activity (DPPH free-RSA assay) in ground red chili pepper

1,1-diphenyl-2-picrylhydrazyl (DPPH) free-RSA test was performed by the method defined by Tripathi & Mishra (2009). One gram of the sample was homogenized in an ultrasonic bath for 15 min with 25 ml of methanol. A 0.5 ml DPPH solution (0.25 mM in 95% methanol) was added to 100 μL of this. It was stirred well and kept in a dark room for 15 min at the room temperature. Blank sample absorption was also prepared and its absorbance was measured immediately. Drop in absorbance was measured at 517 nm by spectrophotometry. Inhibition was calculated by the following formula:

$$\text{Inhibition}(\%) = (\text{Ab} - \text{Aa}/\text{Ab}) \times 100$$

Here, Ab, absorption of blank sample (time = 0 min); Aa, absorption of extract at 15 min.

Elemental analysis in ground red chili pepper

Elemental analyses were done according to the method reported by Karadaş and Kara (2012). Briefly, 0.25 g of sample was placed in 9 ml of concentrated nitric acid and 1 ml of hydrogen peroxide and burned in a microwave. Calcium (Ca), copper (Cu), magnesium (Mg), manganese (Mn), zinc (Zn), iron (Fe), sodium (Na), potassium (K), and cobalt (Co) were determined by inductively coupled plasma mass spectrometry (ICP-MS). Phosphorus (P) was measured according to the method reported by Sulaiman *et al.* (2011). A sample of 2 g was weighed in a beaker and mixed with 4 ml of ammonium molybdate and ascorbic acid. Absorbance of the sample was read at 880 nm after 20 min for color change. The calibration curve was drawn with phosphate standard solutions prepared at seven different concentrations (0.5–2.5 mg/L). Sample concentration was calculated from the standard curve. The accuracy of the methods used was evaluated according to the results obtained from the standard reference material.

Statistical analyses

The normal distribution characteristics of the data were examined by Shapiro–Wilks test. Differences in the regions and AF levels in the variables showing normal distribution were determined by one-way analysis of variance and Duncan's test. The Kruskal Wallis-H test was used to compare groups for data, which did not distribute normally.

Results and discussion

Validation of the method for AFs

The limit of detection (LOD) was 0.13 μg kg^{-1} for AFB1 and AFG1, and 0.04 μg kg^{-1} for AFB2 and AFG2, limit of quantification (LOQ) was 0.4 μg kg^{-1} for AFB1 and AFG1, 0.12 μg kg^{-1} for AFB2 and AFG2. LOD and LOQ were calculated with a signal-to-noise ratio (S/N) 3/1 and 10/1, respectively. The recovery rates of AFs were determined at fortified levels of 1, 4, and 8 μg kg^{-1} in noncontaminated chili pepper in three parallels. The recoveries for AFB1, B2, G1, and G2 were found 89.3, 85.9, 85.4, and 71%, respectively. For all the experiments, the relative standard deviation (RSD) of recovery was $<8\%$ ($n = 3$). The fortified samples of control peppers provided high levels of recoveries of all AFs. The calibration curves of AFB1 and AFG1 were linear between 0.4 and 32 μg kg^{-1}, while for AFB2 and AFG2 were 0.12 and 9.6 μg kg^{-1} ($r = 0.9996$). The retention times of AFB1, AFB2, AFG1, and AFG2 were 11.3, 9.4, 8.0, and 6.5 min, respectively. The method showed good repeatability and intra-laboratory reproducibility.

Validation of the method for capsaicinoids

LOD and LOQ were 5.03 and 16.75 mg kg^{-1} for CAP, and 6.04 and 20.13 mg kg^{-1} for DHCAP, respectively. LOD and LOQ were calculated with a signal-to-noise ratio (S/N) 3/1 and 10/1, respectively. Since it is impossible to find a pepper that does not contain capsaicinoids, the recovery of the method was estimated by spiking with a known amount of each capsaicinoid in 80 ml methanol and subjecting it to the same procedures as the samples, as reported by Manirakiza *et al.* (1999). The recovery rates of capsaicinoids were determined at fortified levels of 10, 200, and 2000 mg kg^{-1} in methanol in three parallels. The recoveries of CAP and DHCAP were 97.5% and 98.7%, respectively. For all the experiments, the RSD of recovery was $<9.3\%$ ($n = 3$). The calibration curves of capsaicinoids were linear between 5 and 2000 mg kg^{-1} ($r = 0.9952$). The method showed good repeatability and intra-laboratory reproducibility.

The frequency of AFs-contamination

AFs were found at measurable levels in 23 of 45 ground red chili pepper samples collected from three different regions (Table 1). The AFB1, AFB2, and AFG1 levels in all samples were determined to be 0.45–48.33 μg kg^{-1}, 0.14–3.62 μg kg^{-1}, and 0.44–0.8 μg kg^{-1}, respectively; while AFG2 was not at a measurable level in any sample. The measurable total AF levels in all samples were found to be 0.45–52.55 μg kg^{-1}. Reddy *et al.* (2001) have shown that total AF levels in chili peppers grown in India can reach up to 969 μg kg^{-1}. We find that these concentrations are much lower than the concentrations in India. AFB1 contaminated chili peppers over the MRL accepted by the European Union and Turkish Food Codex (>5 μg kg^{-1}), was detected in 40% (six samples) of which were from Kahramanmaras samples, 26.67% (four samples) from Gaziantep and 20% (three samples) from Sanliurfa. When total AF contamination level is evaluated, AF contamination over MRL (>10 μg/kg) was not found in any of the pepper samples from Sanliurfa, but found in 20% of samples (three samples from each province) from each other. According to these results, 28.89% of the samples (13 samples) were inconvenient to consume due to AF content.

Table 1. The frequency of aflatoxins in ground red chili peppers.

Aflatoxin	Level	Sanliurfa ($n = 15$)	Kahramanmaras ($n = 15$)	Gaziantep ($n = 15$)	Total ($n = 45$)
Aflatoxin B1	ND[a] (%)	13.34	26.67	20	20
	$<5\ \mu g\ kg^{-1}$ (%)	66.67	33.34	53.34	51.12
	$>5\ \mu g\ kg^{-1}$ (%)	20	40	26.66	28.88
	Range ($\mu g\ kg^{-1}$)	0.51–8.97	0.54–13.13	0.45–48.33	0.45–48.33
Aflatoxin B2	ND (%)	53.34	26.67	33.34	35.56
	Range ($\mu g\ kg^{-1}$)	0.14–0.65	0.14–1.03	0.14–3.62	0.14–3.62
Aflatoxin G1	ND (%)	100	93.34	80	91.12
	Range ($\mu g\ kg^{-1}$)	0	0.51	0.44–0.8	0.44–0.8
Aflatoxin G2	ND (%)	100	100	100	100
	ND (%)	13.34	26.67	20	20
Total aflatoxin	$<10\ \mu g\ kg^{-1}$ (%)	86.66	53.34	60	66.67
	$>10\ \mu g\ kg^{-1}$ (%)	0	20	20	13.34
	Range ($\mu g/kg^{-1}$)	0.51–9.62	0.68–14.67	0.45–52.55	0.45–52.55

[a]ND: not detected.

This rate was similar to the levels of AF contamination and level of nonconformity (27%) found in chili peppers taken from the same regions in 2014 (Özkan *et al.* 2015). Likewise, it is also close to the nonconformity level (22%) of chili pepper, which is obtained from different regions in Turkey (Demircioglu and Filazi 2010). On the other hand, in Turkey, ground red chili peppers sold in Sanliurfa (Karaaslan and Arslanğray 2015), Istanbul (Aydin *et al.* 2007), and Kayseri (Kanbur *et al.* 2006) were contaminated with AFs in rates of 31, 18, and 6%, respectively and reported to be inconvenient for consumption. It has been shown that 18% of the chili red peppers in India (Reddy *et al.* 2001) contain AF above the MRL. The development of toxicogenic fungi in food products and the secretion of AF are influenced by many factors such as moisture content, chemical composition, climate change, harvesting time, temperature, and pH of the sample. Moreover, the difference in the method of analysis can also be the reason for the differences between the studies. Inadequate clean-up or improper drying of harvested peppers increase fungal contamination rates and AF production risk. Therefore, harvested crops should be cleaned and rotten ones should be discarded (Duman 2010).

The nutritional parameters in ground red chili peppers

In the analysis of nutritional parameters, no difference was observed between the cities except the capsaicinoids (Table 2). In another study, the content of CAP and DHCAP in *C. annuum* and *C. frutescens* samples taken from Kahramanmaras and Sanliurfa were measured as 810–1420 and 380–700 mg kg^{-1}, respectively (Poyrazoğlu *et al.* 2005). In terms of capsaicin, these levels are closer to our results, but are lower than the DHCAP content measured in our study (991.2–1044.4 mg kg^{-1}). This may be due to differences in the method of analysis, soil characteristics, or other factors such as planting. The highest content of CAP and DHCAP were measured in the chili peppers produced in Kahramanmaras, followed by those produced in Gaziantep and Sanlıurfa, respectively. It is known that red peppers are hot due to their capsaisinoid ingredients, our results suggest that the bitterest red peppers are grown in Kahramanmaras.

In a study conducted on ripe fruits of some *C. annuum* species grown in Antalya province of Turkey, it was found that 1440–2390 mg kg^{-1} of total carotenoids, 152–649 mg kg^{-1} of ascorbic acid, 11–307.7 mg kg^{-1} of CAP, and 0.1–208 mg kg^{-1} of DHCAP were present (Topuz and Ozdemir 2007). The ratio of ascorbic acid (655.9–728.7 mg kg^{-1}), CAP (1366.1–1503.4 mg kg^{-1}), and DHCAP (991.2–1044.4 mg kg^{-1}) contents measured in our study are much higher than the peppers grown in Antalya.

There was no significant difference between the cities in terms of moisture, ash, crude protein, fat, carbohydrate, crude fiber, metabolic energy, total carotene, RSA, total phenols, and ascorbic acid contents of peppers (Table 2). Our results are similar in the content of moisture, ash, crude protein, crude oil, and crude fiber in the *C. annuum* species grown in the North East region of China (Zou *et al.* 2015). However, the moisture content in the current study was lower than the moisture content in fresh and unflavored *Capsicum* species from Nigeria (Emmanuel-Ikpeme *et al.* 2014); while protein content was higher than those countries. This may be because the peppers were collected in dried form. It is known that high moisture content causes the reproduction of fungi and synthesis of AF. The drying process prolongs the peppers' shelf life (Emmanuel-Ikpeme *et al.* 2014).

In our study, total carotene content was found to be between 1940.4 and 1981.7 mg kg^{-1} on dry matter basis. A study has shown that carotenoids may be present in peppers at a level of 3300 mg kg^{-1} and that

Table 2. Nutritional parameters in ground red chili pepper (Mean ± SD).

Nutritional parameters	Sanliurfa	Kahramanmaras	Gaziantep
Moisture (%)	4.5 ± 0.4	4.4 ± 0.3	4.4 ± 0.4
Ash (%)	5 ± 0.7	4.9 ± 0.3	5 ± 0.5
Crude protein (%)	21.3 ± 1.7	21.3 ± 1.0	21.3 ± 0.3
Fat (%)	17.1 ± 4.7	13.8 ± 4.3	15.2 ± 3.8
Carbohydrates (%)	52.2 ± 4.5	55.5 ± 4.5	54.1 ± 3.8
Crude fiber (%)	17.5 ± 1.9	17.7 ± 1.7	16.9 ± 0.3
Metabolisable energy (kJ kg^{-1})	1861.4 ± 127.4	1818.4 ± 85.3	1846.3 ± 81.2
Capsaicin	1366.1 ± 128.2^{c}	1503.4 ± 112.7^{a}	1464.9 ± 148.2^{b}
Dihydrocapsaicin	991.2 ± 47.7^{c}	1044.4 ± 51.5^{a}	1018.7 ± 57.5^{b}
Total Carotene (mg kg^{-1})	1940.4 ± 137.2	1961.3 ± 90.9	1981.7 ± 82
RSA (%)	84.4 ± 12.4	76.7 ± 18.1	70 ± 24.7
Total phenols (mg/100g)	424.5 ± 50	417.4 ± 64.2	394.6 ± 72.9
Ascorbic acid (mg kg^{-1})	723.1 ± 138.3	728.7 ± 144.5	655.9 ± 198.3
Calcium (μg g^{-1})	2490 ± 290	2570 ± 300	2550 ± 340
Cobalt(μg g^{-1})	512.1 ± 13.9	519.5 ± 16.9	515.7 ± 13.9
Copper (μg g^{-1})	11.6 ± 0.4	11.7 ± 0.4	11.8 ± 0.5
Iron (μg g^{-1})	340 ± 13.8	336.8 ± 15.3	346 ± 11.9
Potassium (μg g^{-1})	17.9 ± 0.25	18 ± 0.26	18.1 ± 0.21
Magnesium (μg g^{-1})	1790 ± 130	1770 ± 170	1790 ± 120
Manganese (μg g^{-1})	17.4 ± 0.3	17.6 ± 0.4	17.6 ± 0.4
Sodium (μg g^{-1})	132.1 ± 7.0	131.1 ± 5.8	130.7 ± 5.5
Phosphorus (μg g^{-1})	1250 ± 170	1290 ± 180	1270 ± 140
Zinc (μg g^{-1})	32.8 ± 1.6	32.3 ± 1.5	32.4 ± 1.6
Na/K	7.4	7.3	7.2
Ca/P	1.99	1.99	2
K/(Ca + Mg)	0.004	0.004	0.004

abc: Within lines, the means followed by the different letter are significantly different ($p < 0.05$).

the pigment content of chili peppers may decline considerably even if they are kept in the dark and at appropriate temperatures for 4 months (Schweiggert *et al.* 2007). Carotenoids are responsive to oxidation and degradation in response to environmental conditions. High relative humidity causes enzymatic hydrolysis (Tripathi and Mishra 2009). In our study, it is not known under what conditions and for how long the peppers were kept before the samples were taken. The reason for the difference may be inadequate storage conditions and durations. When the element content of the collected peppers were examined, no differences were found between cities. These levels were similar to Zn, Mg, Fe, Mn, Cu, Ca, and Co values determined in chili peppers collected from Balıkesir province of Turkey (Karadaş and Kara 2012). On the other hand, our samples contained higher Na and Mn, but lower K ingredient than the *Capsicum* species grown in Tenerife Island (Rubio *et al.* 2002). Ash and mineral contents indicate that pepper can be a good source of valuable minerals. The most abundant elements in peppers are Ca, and in the decreasing order of Ca > Mg > P>Co > Fe > Na > Zn > K>Mn > Cu. However, it is reported that the element present in highest amount in peppers is K (Park *et al.* 2006, Zou *et al.* 2015). This may be the result of the use of potassium-containing fertilizer (Iqbal *et al.* 2017). Ca is an element essential for the function of the nerves, the normal movements of the muscles, blood coagulation, cardiac function, and cell metabolism. In addition, Ca is necessary for the hardness of bones and teeth, together with P. The Ca/P ratio was found approximately 2 in all samples examined. According to a previous study (Aremu *et al.* 2011), a foodstuff is good if the Ca/P ratio is more than 1, but not adequate if it is less than 0.5. Thus, the values found in this study were evaluated as good. In the same way, to protect against hypomagnesemia, the ratio of K/(Ca + Mg) in the diet should be less than 2.2. (Aremu *et al.* 2011). The K/(Ca + Mg) ratio in the peppers we analyzed was 0.004. Thus, consumption of these red pepper flakes does not cause hypomagnesemia. Under normal conditions, the Na/K ratio in the peppers is less than 1 (Ogunlade *et al.* 2012). However, the Na/K ratio of all the peppers we have analyzed was found higher than 7. This is because of the traditional addition of salt in order to increase the durability of the peppers, as they are dried and pulverized. That is why caution should be paid when peppers are used as spices in the preparation of diets for hypertensive patients. Minerals are important nutritional sources for many plants and they participate in a number of biological activities in the human body. Minerals found in chili peppers can cover some of our daily needs. For this reason, it can be said that the peppers are important contributors to health due to the elements they contain. When assessed for all nutritional parameters, differences observed by country or

region may be associated with individual genetic variations or environmental factors in plants. The content and composition of the secondary metabolites in plants can be affected by the genetic structure of plants, soil characteristics, environmental factors, and agricultural practices, especially the plant variety. In addition to these factors, post-harvest applications also have significant effects on these compounds. Even when the same plant species are grown under the same conditions, they may gather nutrients in different amounts from the soil (Yaldiz *et al.* 2010).

Nutritional parameters based on AF content

In order to understand the relationship between AFs and nutritional parameters of ground red chili peppers, they are categorized into three as: (1) those containing AFB1 at non-measurable levels (<LOD), (2) those containing measurable levels of AFB1 but acceptable levels of AF according to the MRL (<5 μg kg^{-1}), and (3) those containing AFB1 over MRL (>5 μg kg^{-1}) and these groups were compared in terms of nutritional parameters (Table 3). There was a decrease in the content of fat, crude fiber, metabolic energy, RSA, total phenol, and ascorbic acid; while there was an increase in the content of carbohydrates in the third group containing AFB1 over MRL levels compared to others two groups. There was no statistical difference in element levels between groups, therefore not shown again. Most of these parameters are also important quality criteria for pepper exports and consumption. Thus, Table 3 provides important information about the relationship between naturally occurring AFs and the quality of peppers. In an experimental study (Tripathi and Mishra 2009), it was reported that *A. flavus* causes an increase in the total protein content, but decrease in capsaicin and total sugar contents in powdered red peppers. In contrast, in our study, AFs did not change the crude protein content of red peppers. It is known that in the method used to measure crude protein content, the actual protein value of the sample cannot be obtained accurately and the error can occur, because the substances that do not have actual protein properties are taken into account in the calculation. In order to find the true protein content, determine the total amino acids need to be determined. However, we could not afford to carry out the analyses in our study due to high cost and laborious process. In our study, we showed that AF-contamination decreased the oil content of the red peppers from 16.5 to 12.4% (25% loss). However, this loss is less than the 82.31% loss in oil content reported by Tripathi and Mishra (2009). All these differences may be due to differences in the methods used or the difference in the AFB1 producing fungi. Consequently, AF production has resulted in a reduction of oil content in pepper flakes.

Likewise, in our study, we found that the frequency of AF contamination is higher in chili peppers containing the highest concentration of capsaicin. In an *in vitro* study (Teel 1991), capsaicin was reported to reduce the binding of AFB1 to DNA and decrease the adduct formation. Capsaicin significantly reduces AFB1 binding to bovine thymus DNA and AF-DNA adduct (AFB1-N^7-Gua) formation, in a dose-dependent pattern. In the same study, it has also been shown that capsaicin reduces liver enzyme activity and thus inhibits AFB1 biotransformation. For this reason, AF-contaminated but not bitter peppers may contain lower amounts of capsaicinoids, thus may be more dangerous when consumed. Therefore, it is suggested that Kahramanmaras peppers containing the highest rates of AF compensate these negativities due to their high capsaicinoids content.

The use of carotenoids in the control of growth of AF-producing fungi is still controversial. Norton (1997)

Table 3. Nutritional parameters by Aflatoxin B1 (Mean ± SD).

Nutritional parameter	<LOD ($n = 9$)	<5 μg kg^{-1} AFB1 ($n = 23$)	>5 μg kg^{-1} AFB1 ($n = 13$)
Moisture (%)	4.25 ± 0.35	4.51 ± 0.38	4.43 ± 0.29
Ash (%)	4.99 ± 0.48	4.9 ± 0.59	5.05 ± 0.29
Crude protein (%)	21.5 ± 1.15	21.2 ± 1.2	21.3 ± 1.09
Fat (%)	16.5 ± 4.5[a]	16.6 ± 4.2[a]	12.4 ± 3.2[b]
CHO (%)	52.7 ± 4.9[b]	52.7 ± 4.0[b]	56.8 ± 3.6[a]
Crude fiber (%)	19.3 ± 0.6[a]	17.8 ± 0.9[b]	15.3 ± 2.14[c]
Metabolisable energy (kJ/kg)	1873.7 ± 92[a]	1860.9 ± 109[ab]	1786.6 ± 63.5[b]
Capsaisin (mg kg^{-1})	1405.4 ± 121.3	1422 ± 141.9	1512.4 ± 135.4
Dihydrocapsaicin (mg kg^{-1})	1014.4 ± 53.5	1005.1 ± 48.1	1043.6 ± 64.7
Total Caroten (mg kg^{-1})	1926.7 ± 99.7	1950.8 ± 108.8	2003.4 ± 95.8
RSA (%)	92.7 ± 2.5[a]	83.5 ± 11.6[a]	54.9 ± 18.9[b]
Total phenols	463.4 ± 35.9[a]	431.7 ± 32.9[a]	342.1 ± 60.6[b]
Ascorbic acid (mg kg^{-1})	829.2 ± 29.2[a]	753.1 ± 95.8[a]	525.3 ± 168.2[b]

ab: Within lines, the means followed by the different letter are significantly different ($p < 0.05$).

argues that the growth of *A. flavus* is not affected by carotenoids. Conversely, Capsanthin (Masood *et al.* 1994), and capsantal (a commercial product containing red pepper extract, ethoxyquin and excipient) (Santos *et al.* 2010) were suggested to prevent the growth of *A. flavus*. However, Santos *et al.* (2010) showed that although capsantal inhibits the growth of *A. flavus*, it does not affect AF production and AF production depends on temperature and time. In our study, no such relationship was found between the AFs and the carotenoids (capsaicinoids and total carotenes) in the samples (Table 3). Conversely, the frequency of AF contamination was highest in peppers containing the highest concentration of capsaicin. So, as claimed by Santos et al (2010), the AF accumulation in the samples may have been influenced by external factors (temperature and time) rather than internal factors. Thus, it was concluded that the growth of fungi and AF production can be limited if the peppers are stored at normal industrial storage temperatures (10 °C).

The reduction of ash content in the corn (Aziz et al, 2000) and red peppers (Tripathi and Mishra 2009) by *A. flavus* were not observed in our study. It is known that a reduction in ash content causes a reduction in mineral content. However, in the current study, the ash content was not affected by AFs, thus the element contents did not change according to AF contamination. On the contrary, Aziz *et al.* (2000) claimed that *A. flavus* consumes Zn, Cu, and Fe contents in corn. This may arise from differences between plant types or methods of analysis.

In our study, the ascorbic acid contents of peppers decreased by 36.6% due to the effect of AFs (Table 3). Nevertheless, there was less loss in our study than the result reported by Tripathi and Mishra (2009). It is known that ascorbic acid is sensitive to light, oxidation, and temperature. In addition, AF-producing fungi secrete phenol oxidases that facilitate ascorbic acid oxidation. The loss of ascorbic acid may have been found to be lower in our study since it is lost during fungi reproduction or drying. It is also thought that this also causes the decrease in total phenols.

The RSA content was found to be quite high (92.7%) in the ground red chili peppers, which have no detectable AFs. It is known that free RSA, measured by DPPH analysis, dependents on the ability of antioxidants to give hydrogen. Antioxidant activity in plants is related to their phenol, β-carotene, and ascorbic acid contents (Mohd Zin *et al.* 2006). However, since most of the biochemical parameters were lost due to AF, the quality of the peppers deteriorated and RSA decreased to 54.9%. The same effect was observed by Ogunlade *et al.* (2012) and Tripathi and Mishra (2009).

Conclusion

Turkey is one of the most pepper cultivation performing country in the world. However, there are a limited number of researches exploring the quality parameters important for pepper exports and consumption. In addition, previous studies on the fungi production and secretion of AFs in ground red chili peppers and their effects on nutrient contents were carried out under experimental conditions. This work is believed to be the first study investigating how fungi reproduced in natural conditions affect biochemical contents of red peppers by AF synthesis. Variations between the contaminated and uncontaminated specimens suggest that some nutritional parameters of the red peppers were affected by AFs. Apart from public health, some of these components are important quality criteria for exports of red peppers. Although the peppers grown in Turkey contain high protein, RSA, ascorbic acid, and element ingredients, exports are low due to AFs. The inability to manufacture in accordance with international standards and consumer preferences naturally reduces the chance of competition in other markets. For this reason, modernization of red pepper enterprises and factories is essential and technical-hygienic conditions should be improved to meet the needs and demands of our modern and developed societies.

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