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Investigation of multimycotoxins by LC-MS/MS in maize semolina chips

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Abstract: Chips made from maize semolina are rarely mycotoxin analysed because they are classified as low-risk foods in routine legal control plans. It is essential to foresee the health risks these snack foods may pose in the medium and long term, whose consumption frequency and quantity have increased with the changing consumer behaviours during the pandemic. The most outstanding development in mycotoxin analysis in recent years has been the use of high-pressure liquid chromatography together with mass spectrometry (LC-MS/MS) as a detector. For aflatoxin (AF) B_1, B_2, G_1, G_2, ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), citrinin (CIT), HT-2 toxin, and T-2 toxin determination in our samples, the LC-MS/MS analysis method with electrospray ionisation interfaces was utilised. Aflatoxin B_1 levels in 22.7% of the samples (2.01–17.49 μg·kg^{-1}) and total aflatoxins (TAF) in 26.7% of the samples (6.71–24.67 μg·kg^{-1}) were determined to exceed the limits defined in the Turkish Food Codex Contaminants Regulation. CIT could not be detected in any of the samples. ZEN + DON + OTA was found in 21.3% of the samples, DON + TAF + total fumonisins (FUM) in 19.3%, and TAF + ZEN + FUM in 18.7%.

Keywords: contamination; corn; food safety; mycotoxin; snacks; liquid chromatography; mass spectrometry

There have been significant changes in people's food consumption habits due to social restrictions and full closure decisions caused by COVID-19. Chips consumption by individuals increased by 33% in this period (Household Consumption Panel 2020). In addition to health-risk components such as high energy, fat, saturated fat, trans fat, sodium and direct sugar content, mycotoxin contaminants in chips should not be ignored (WHO 2002).

Toxic secondary metabolites produced by various fungal species in food and feed are highly alarming. These metabolites cause severe effects on human and animal health. Even at low concentrations, long-term exposure to some mycotoxins can affect the body's fundamental

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functions, leading to liver damage and immune system disorders (Udomkun et al. 2017). Also, in cases of extensive exposure, they cause neurotoxicity, dermatotoxicity, carcinogenicity, mutagenicity, estrogenicity, and teratogenicity (Beltrán et al. 2009; Copetti et al. 2012). Grains are susceptible to mycotoxin contamination due to environmental factors such as high temperatures, high relative humidity, sunlight, insect damage, other pest attacks, and improper storage conditions. Fungal activity and mycotoxin production can render grain unusable for food or feed. If the grain is stored at a moisture content of ≤ 0.70 a_w, no deterioration will occur. However, the investment and increased energy expenditures to ensure this level in silos are seen as an unbearable operating cost burden. Climate changes make it challenging to achieve these conditions and encourage the formation of fungi and mycotoxins, rendering the problem inevitable (Paterson and Lima 2010; Suleiman et al. 2013).

Maize is one of the most widely grown cereals in the world due to its diversity of uses as both food and feed. In the marketing season of 2020–2021, a total of 1.146 billion tonnes of maize were produced worldwide (BUGEM 2021). Maize can be used by separating into relatively pure chemical compounds such as starch, protein, oil, and fibre and by reducing the particle size while preserving some or all of the corn germ and fibre content (Johnston et al. 2005). The first product of the process applied to reduce the particle size is flaking semolina; they consist of large pieces of endosperm and are used primarily in breakfast cereals, and a variety of human foods such as baked goods and chips (Robens and Cardwell 2003). Even if chips are produced at high temperatures, mycotoxins pose a health risk due to their heat resistance (Palumbo et al. 2020). For this reason, controlling these risky snacks is of great importance regarding food safety and consumer health protection. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the only technique to analyse these secondary fungal metabolites with diverse physicochemical properties in a single study. This method is viable for the multi-mycotoxin detection of cereal and cereal products (Amirahmadi et al. 2018).

Several studies have been conducted on maize matrix in South Korea (Kim et al. 2017), Egypt (Abdallah et al. 2017), Iran (Amirahmadi et al. 2018), South Africa (Tebele et al. 2020), Brazil (Franco et al. 2019), Kenya (Kagot et al. 2022), European, African, Asian, and South American countries (Raj et al. 2022). This study aimed to determine multi-mycotoxins' presence in commercially available maize semolina chips with the LC-MS/MS technique.

MATERIAL AND METHODS

Sample collection. This study collected 150 maize semolina chips in their original packages (20–205 g) from local and national markets in Istanbul, Türkiye, from July 2021 to April 2022. The samples which were kept in dry conditions by preserving the integrity of the packaging were immediately analysed.

Preparation of mobile phases. The extraction solution was prepared by mixing 800 mL of methanol (Merck, Germany) with 200 mL of LC-MS grade water (Merck, Germany). The mobile phases were constituted of 0.252 g of ammonium formate in 1 L of LC-MS grade water containing 1 mL formic acid (mobile phase A) and 0.252 g of ammonium formate in 1 L of LC-MS grade methanol containing 1 mL formic acid (mobile phase B).

Sample preparation and extraction. The samples were homogenised in the grinder (Retsch GM 200, Haan, Germany). Five g of the samples were weighed. The weighed samples were mixed with 12 mL of the extraction solution for 2 h while shaken. At the end of the period, the samples were centrifuged at 3 000 g for 5 min, passed through a 0.45 µm filter, and collected in a 15 mL centrifuge tube. The filtrate was transferred into a 1.5 mL vial with a volume of 800 µL. After adding 200 µL of mobile phase A, the solution was analysed. Two replicates were performed in one day.

LC-MS/MS equipment. LC-MS/MS analysis was performed using an Agilent 6420 Triple Quad/G6420A LC-MS system. Chromatographic separation was performed on an Athena C18-WP column (CNW Technologies) (100, 50 mm × 2.1 mm × 3 µm particle size) at a column temperature of 35 °C. A gradient program was set up as follows: 0–3 min with 25% B, 3.0–5.0 min linear gradient down to 70% B; hold at 100% B for 2 min; return to 0% B in 0.1 min (total run time 7.1 min). The flow rate was 0.35 mL·min^{-1}, and the injection volume was 20 µL. The LC flow was directed into the MS detector between 1.0 and 7.0 min. Delta EMV for MS analysis: 400 (+), 400 (–); gas temperature: 350 °C; gas flow: 11 L·min^{-1}, Nebulizer: 30 psi and Capillary: 3 500 V (+), 2 500 V (–). For optimised parameters see Electronic Supplementary Material (ESM), Table S1.

Recovery rate. Mycotoxin standards of aflatoxin AFB_1, AFB_2, AFG_1, AFG_2, ochratoxin A (OTA), fumonisin FB_1, and FB_2 were purchased from Pa group (Ankara, Türkiye); deoxynivalenol (DON), zearalenone (ZEN), citrinin (CIT), T-2, and HT-2 were from Trilogy (Washington, USA) in liquid form 1 000 mg·L^{-1},

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they were stored at 4 °C. The standard solutions for AFB_1, AFB_2, AFG_1, AFG_2, OTA were diluted 0.5 – 1 – 2.5 – 5 – 10 mg·L^{-1}; for ZEN, HT-2 toxin, T-2 toxin, CIT 5 – 10 – 25 – 50 – 100 mg·L^{-1}; for FB_2 25 – 50 – 125 – 250 – 500 mg·L^{-1}; for DON and FB_1 50 – 100 – 250 – 500 – 1 000 mg·L^{-1}.

The LC-MS/MS method was validated to investigate performance characteristics such as linearity, the limit of detection (LOD), the limit of quantification (LOQ), and accuracy, according to the European Commission (2002). Linear regression analysis was performed for a standard mycotoxin mixture of AFB_1, AFB_2, AFG_1, AFG_2, FB_1, FB_2, T-2, HT-2, OTA, DON, and ZEN under optimised LC-MS/MS conditions. External calibration was performed. To ensure the peak measurements' accuracy, we ensured the signal area was at least three times larger than the noise area.

The LOD and LOQ studies used bread, wheat flour, maize flour, and maize semolina chips. We conducted a blank sample analysis to verify that there were no mycotoxins present in the samples. Upon observation, 100 µg·kg^{-1} for DON; 0.3 µg·kg^{-1} for AFB_1 and AFB_2; 0.4 µg·kg^{-1} for AFG_1 and AFG_2; 0.8 µg·kg^{-1} for OTA; 25 µg·kg^{-1} for FB_1; 60 µg·kg^{-1} for FB_2; 100 µg·kg^{-1} for CIT; 10 µg·kg^{-1} for ZEN, T-2, and HT-2 spiking levels were performed. Spiking was performed to determine LOD and LOQ. This study was conducted by two technicians (also to put forth the analysis competencies). Ten measurements with two replicates were taken for each mycotoxin in matrices. The measurement data's standard deviation (SD), LOD, and LOQ, were calculated using specific formulas, Equations 1–3.

$$SD = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(xi - xmean\right)^2} \quad (1)$$

$$LOD = SD \times 3 \quad (2)$$

$$LOQ = SD \times 10 \quad (3)$$

where: LOD – limit of detection; LOQ – limit of quantification; SD – standard deviation; *xmean* – arithmetic mean of data; *xi* – each value of dataset; *n* – total number of data.

ISO 2010 (International Organization for Standardization ISO/TS 21748 Guidance for the Use of Repeatability, Reproducibility and Trueness Estimates in Measurement Uncertainty Estimation) and Ellison & Williams 2012 references were used to determine measurement uncertainties. Repeatability, reproducibility, and uncertainty budgets from recovery were combined as indicated in the references. Uncertainty budgets for each active ingredient were calculated separately. The repeatability, reproducibility and recovery uncertainty budgets included uncertainty about the equipment used. LOD, LOQ, recovery, and standard uncertainty ratios are provided in ESM, Table S2, correlation coefficient R^2 and regression equations in ESM, Table S3.

RESULTS AND DISCUSSION

The result of the study revealed that 22.7% of the samples exceeded the maximum limits specified in the Turkish Food Codex Contaminants Regulation in AFB_1 (x: 2.15 ± 2.10 µg·kg^{-1}) and 26.7% exceeded the TAF limits (x: 4.21 ± 4.33 µg·kg^{-1}). It was determined that 64% of the samples were contaminated with AFB_1 (range: 0.89–17.49 µg·kg^{-1}), 56.7% with AFB_2 (range: 0.82–3.02 µg·kg^{-1}), 46% with AFG_1 (range: 0.75–2.62 µg·kg^{-1}), and 44% with AFG_2 (range: 0.79–12.75 µg·kg^{-1}) at levels above the detectable limits. While this rate was 28% for FB_1 (range: 141.98–157.78 µg·kg^{-1}), contamination with FB_2 (range: 57.22–198.26 µg·kg^{-1}) was detected in all samples. Furthermore, 86.7% of the samples were contaminated with OTA (range: 1.30–1.31 µg·kg^{-1}), 64% T-2 (range: <10.55 µg·kg^{-1}), 42.7% HT-2 (range: 14.59–44.98 µg·kg^{-1}), and 22% ZEN (range: 16.03–17.33 µg·kg^{-1}) and DON (range: 167.91–176.25 µg·kg^{-1}). CIT values were below the LOD in all samples (Table 1).

Maize, an essential grain for animal and human nutrition, draws attention with its wide variety of snack alternatives today. In the literature, no study targets only maize semolina chips. The studies carried out in the last five years on maize matrix can be summarised as follows.

Kim et al. (2017) identified 13 mycotoxins in South Korean maize kernels by LC-MS/MS. The incidences of AFB_1, FB_1, FB_2, T-2, DON, and ZEN in maize kernels have been reported at 1%, 47%, 59%, 2%, 13%, and 7%, respectively. In positive samples, AFB_1 5.2 µg·kg^{-1}, FB_1 3.8–2 990 µg·kg^{-1}, FB_2 1.9–620 µg·kg^{-1}, T-2 6.4–13.7 µg·kg^{-1}, DON 17–1 405 µg·kg^{-1}, ZEN 0.9–14.7 µg·kg^{-1} concentrations were determined. AFB_2, AFG_1, AFG_2, HT-2, and OTA could not be detected in any samples.

Abdallah et al. (2017) reported that 79 maize samples collected from Egypt were contaminated with at least four toxins. They reported that the transmission rate

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other than AFB_1 is not threatening, according to international standards. The prevalence of AFB_1 was 16%, and the median of positive samples was 4.81 μg·kg^{-1}.

A study was conducted on Iran's gristmill maize flour samples by Amirahmadi et al. (2018). In that study, AFB_1 was found in 76.6% of the samples; OTA was detected in 20% and ZEN in 46%. The mean contamination was measured as 154.1 μg·kg^{-1}, 25 μg·kg^{-1}, and 358.7 μg·kg^{-1}, respectively. Co-occurrence of AFB_1 + ZEN in 20% and FB_1 + OTA + ZEN was detected in 23% of the maize samples. It has been reported that the measured contamination level for DON and T-2 toxin of maize flour samples was below the maximum tolerated level.

A study evaluated the co-occurrence of mycotoxins in samples of maize food (n = 26) collected from small-scale farms in Brazil (Franco et al. 2019). The median levels of mycotoxins found in maize foods were determined as 2.5 μg·kg^{-1} (TAF), 120 μg·kg^{-1} (FUM), 13 μg·kg^{-1} (ZEN), and 57 μg·kg^{-1} (DON). Except for the FUM in a maize meal sample, all values were below the Brazilian tolerance limits. The co-occurrence of two or more mycotoxins in maize food was 35%.

Tebele et al. (2020) reported that they detected ten types of mycotoxins, including α-ZEL (79%), FB_1 and FB_3 (92%), OTB (54%), DON (21%), OTA (8%), and AFB_2 (4%) in maize samples in a study conducted in South Africa. Unusually, none of the samples analysed had AFB_1, AFG_1, and AFG_2. AFB_2 was recovered from only one maize sample (0.21 μg·kg^{-1}). ZEN and its derivative β-ZEL were not detected in any samples, unlike α-ZEL detected in maize. The data revealed the concentration range for α-ZEL was 6.5–49 μg·kg^{-1}. Ochratoxins levels from all samples analysed in that study were below the LOQ. CIT, T-2, and HT-2 were not detected in any of the samples examined. All mycotoxins detected in maize, except FB_1 and FB_3, were within the maximum regulatory limits of South Africa and the European Commission, and the highest FB_1 concentration was 2 153 μg·kg^{-1}.

Kagot et al. (2022) evaluated the mycotoxin levels in maize from Kenyan households (n = 480). The highest AF contamination was found in Eastern Kenya maize samples. Out of the samples tested, it was discovered that 75% had AF levels exceeding the regulatory limits set in Kenya (10 μg·kg^{-1}). The maximum level recorded was 558.1 μg·kg^{-1}. In Western Kenya, only 18% of samples were reported to have concentration levels for AF above the Kenyan regulatory limits (max. 73.3 μg·kg^{-1}). It was found that 20% of the samples exceeded the Kenyan regulatory limit for FUM (2 000 μg·kg^{-1}) (max. 13 022 μg·kg^{-1}). In addition, 21.6% of the samples taken from the Lake Victoria region were above the European regulatory limits (1 000 μg·kg^{-1}) for ZEN and DON. It was determined that the western region had the most minor AF contamination (18%), while the Eastern region had the highest (81%).

Mycotoxin contamination of maize was studied in different global regions (Europe, Africa, Asia, and South American countries) over three years (2018–2020). Of the more than 1 000 samples analysed each year, > 75% were found to be multi-contaminated with different mycotoxins. Trends in mycotoxin contamination across all four sites showed contamination consistent with DON in 3 sampling years. AFB_1 pollution was widespread in all regions in 2018. Nevertheless, in 2019 it was dominant in Europe. It was concluded that maize FB_1 contamination in Europe in 2018 became more prevalent in Asian and Latin American countries in 2019 and African maize in 2020 (Raj et al. 2022).

Many countries and international organisations have enacted crucial regulations on 'acceptable health risk' to control aflatoxins and prohibit the trade of contaminated foods. These regulations generally depend on a country's level of economic development, the rate of consumption of high-risk products, and the susceptibility of crops to contamination (Kendra and Dyer 2007; Udomkun et al. 2017). The safe levels of AF for human consumption have been determined between 4–30 μg·kg^{-1}. The EU stated that no direct human consumption product should be present at levels higher than 2 μg·kg^{-1} for AFB_1 and more than 4 μg·kg^{-1} for total aflatoxins (EC 118 2006; EC 165 2010).

Although the sample rate exceeding the AFB_1 legal limit values was 22.7% (n = 34) in our study, this rate was calculated as 44.7% (n = 67) when the samples with > 1 μg·kg^{-1} concentration of AFB_1 were included in the risky group that could cause accumulation in the organism (Table 1). AFs are classified in Group 1, whose genotoxic and carcinogenic effects for humans have been revealed (IARC 1993; Bennett 2003). AFB_1 is acutely and chronically most prevalent and toxic of all AFs. AFB_1 is associated with a high incidence of hepatocellular carcinoma, and regions more exposed to AF have been reported to have a higher disease prevalence (IARC 1993; Groopman 2011). Some clinical findings, including vomiting, anorexia, other gastrointestinal symptoms, pulmonary oedema, depression, weight loss, haemorrhages, and liver necrosis, have been linked to exposure to aflatoxins (EFSA 2004; Groopman 2011). Due to these toxic

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Table 1. Descriptive statistical data of all samples (total n = 150)*

Mycotoxin	LOD > (µg·kg^{-1})	LOD – LOQ > (µg·kg^{-1})	Concentration (µg·kg^{-1})			x (µg·kg^{-1})	SD (µg·kg^{-1})	Med (µg·kg^{-1})
	n	n		n				
AFB_1	< 0.24	0.24–0.78 >	≥ 0.78–1	≥ 1–2	≥ 2	2.15	2.10	1.89
	54	23	6	33	34			
AFB_2	< 0.26	0.26–0.82 >	≥ 0.82–1	≥ 1–2	≥ 2	2.31	0.34	2.33
	65	51	1	1	32			
AFG_1	< 0.22	0.22–0.74 >	≥ 0.74–1	≥ 1–2	≥ 2	2.31	0.38	2.38
	81	36	1	1	31			
AFG_2	< 0.24	0.24–0.78 >	≥ 0.78–1	≥ 1–2	≥ 2	2.07	1.67	1.80
	84	17	6	6	37			
TAF	< 0.22	0.22–0.74 >	≥ 0.74–2	≥ 2–4	≥ 4	4.21	4.33	1.65
	0	50	49	11	40			
FB_1	< 31.14	31.14–103.79 >	≥ 103.79–150	≥ 150–200	≥ 200	146.17	7.75	142.47
	108	38	3	1	0			
FB_2	< 17.11	17.11–57.04 >	≥ 57.04–100	≥100–200	≥ 200	109.04	62.08	61.12
	0	34	67	49	0			
FUM	< 17.11	17.11–57.04 >	≥ 57.04–200	≥ 200–800	≥ 800	117.04	64.35	62.45
	1	20	125	4	0			
OTA	< 0.26	0.26–0.85 >	≥ 0.85–1	≥ 1–3	≥ 3	1.3	< 0.1	1.3
	20	96	0	34	0			
DON	< 28.51	28.51–95.03 >	≥ 95.03–150	≥ 150–500	≥ 500	171.3	2.6	170.6
	117	0	0	33	0			
ZEN	< 2.94	2.94–9.80 >	≥ 9.80–50	≥ 50	≥ 50	16.41	0.34	16.32
	117	0	33	0	0			
HT-2	< 3.32	3.32–11.08 >	≥ 11.08–20	≥ 20–40	≥ 40–50	35.17	8.65	38.91
	86	23	7	28	6			
T-2	< 3.16	3.16–10.55 >	≥ 10.55	≥ 10.55	≥ 10.55	LOQ >	LOQ >	LOQ >
	54	96	0	0	0			
CIT	< 30.44	30.44–101.5 >	≥ 101.5	≥ 101.5	≥ 101.5	LOD >	LOD >	LOD >
	150	0	0	0	0			

* when calculating x, SD, and Med, only values equal to and above the LOQ are included; AFB_1 – aflatoxin B_1; AFB_2 – aflatoxin B_2; AFG_1 – aflatoxin G_1; AFG_2 – aflatoxin G_2; CIT – citrinin; DON – deoxynivalenol; FB_1 – fumonisin B_1; FB_2 – fumonisin B_2; HT-2 – HT-2 toxin; OTA – ochratoxin A; TAF – total aflatoxins; FUM – total fumonisins; T-2 – T-2 toxin; ZEN – zearalenone; LOD – limit of detection; LOQ – limit of quantification; SD – standard deviation; x – mean; Med – median; n – number of samples

properties, a safe dose threshold dose has not been determined.

In our study, although no samples with FUM, OTA, and ZEN concentrations exceeded the legal limit values, the sample rate with FB_1 and FB_2 above the detection limits was calculated as 28% and 100%. OTA detected sample rate was 86.7%, and ZEN detected sample rate was 22%. FB_1 and FB_2 are the two most prevalent fumonisins. FB_1 is considered the most toxic fumonisin and is classified by IARC as possibly carcinogenic to humans (Group 2B) (IARC 1993). Although inconclusive, FUM, particularly FB_1, have been linked to human oesophagal cancer (Bennett 2003). Fumonisin contaminations typically occur before harvest or at the beginning of storage (Marin et al. 2013), and nearly all contaminations originate from maize (EFSA 2014). Despite the high prevalence of fumonisins in maize samples, their concentration does not

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Table 2. The co-occurrence of two types of mycotoxins (*n*)*

AFB_1	**AFB_2**	**AFG_1**	**AFG_2**	**FB_1**	**FB_2**	**OTA**	**DON**	**ZEN**	**HT-2**	**T-2**
AFB_1	42	40	38	11	79	77	33	41	43	33
–	**AFB_2**	51	57	29	63	63	33	56	63	33
–	–	**AFG_1**	46	18	51	46	32	46	51	0
–	–	–	**AFG_2**	27	57	57	30	50	57	29
–	–	–	–	**FB_1**	32	32	0	24	30	0
–	–	–	–	–	**FB_2**	98	33	57	64	33
–	–	–	–	–	–	**OTA**	33	57	64	33
–	–	–	–	–	–	–	**DON**	32	33	32
–	–	–	–	–	–	–	–	**ZEN**	52	32
–	–	–	–	–	–	–	–	–	**HT-2**	33
–	–	–	–	–	–	–	–	–	–	**T-2**

* samples above the LOD values; AFB_1 – aflatoxin B_1; AFB_2 – aflatoxin B_2; AFG_1 – aflatoxin G_1; AFG_2 – aflatoxin G_2; FB_1 – fumonisin B_1; FB_2 – fumonisin B_2; OTA – ochratoxin A; DON – deoxynivalenol; ZEN – zearalenone; HT-2 – HT-2 toxin; T-2 – T-2 toxin

increase during storage (Marin et al. 2013), making them relatively simple to control. OTA can be found in various matrices, particularly in poorly dried cereals and cereal products (Belli et al. 2002). According to reports, the half-life of OTA in the human body is 35 days after ingesting a food bite (Studer-Rohr et al. 2000). *In vivo* experiments revealed that OTA accumulates in the kidneys. Therefore, its main toxic effect is nephrotoxicosis. Exposure to OTA has also been associated with carcinogenicity, teratogenicity, immunotoxicity, and neurotoxicity (EFSA 2006). IARC identified OTA as a human carcinogenic compound in Group 2B (IARC 1993). The most crucial feature of ZEN, which has a very stable structure against heat, is the similarity of its chemical structure to estrogens (Marin et al. 2013; Gzyl-Malcher et al. 2017). It can destroy reproductive system germ cells, genital dysfunction, stillbirths, and even infertility. In addition, ZEN can cause hepatotoxicity, haematological toxicity, immunotoxicity, and genotoxicity and is associated with the development of estrogen-dependent cancers such as breast cancer (Ahamed et al. 2001; Gzyl-Malcher et al. 2017). Milk retention or absence in women, rectal prolapse, low testosterone levels and spermatogenesis in men are additional clinical manifestations of hyperestrogenism (Marin et al. 2013).

On the other hand, it is essential to evaluate their co-occurrence due to the synergistic adverse effects of co-occurring mycotoxins on human and animal health (Smith et al. 2016). Some foods may contain multiple mycotoxins, but most studies have focused on forming a single mycotoxin. Legal regulations do not consider the combined effects of mycotoxins. However, many studies have been conducted on the natural co-occurrence of mycotoxins with AFs, OTA, ZEN, FUM, and trichothecenes (TCTs), especially DON. AFs & FUM, DON & ZEN, AFs & OTA, and FUM & ZEN are mostly observed in cereals and grain products samples. Few studies have reported the number of co-occurring mycotoxins, the proportion of contaminated samples, and the most prevalent mycotoxin combinations (Smith et al. 2016). In our research, the co-occurrence rates of 2 mycotoxins in the samples are given in Table 2. ZEN + DON + OTA was found in 21.3% of the samples, DON + TAF + FUM 19.3%, and TAF + ZEN + FUM in 18.7% (Figure 1).

Climatic conditions such as relative humidity and temperature affect maize fungal infestation and maize contamination with mycotoxins in pre- and post-harvest periods (Hawkins et al. 2005; Channaiah and Maier 2014). The freshly harvested, shelled maize cobs are traditionally spread out on the ground and exposed to the sun to reduce their moisture content. In this process, the changes in weather conditions, the prolongation of the drying period and the damage of the grains by the effect of agricultural pests can be considered risk factors in terms of mycotoxins (Suleiman et al. 2013). Errors in stacking and ventilation during storage and transportation processes and unfavourable storage conditions such as high temperature and high relative humidity can be considered factors that increase the mycotoxin concentration of the raw material (Hawkins et al. 2005; Channaiah and Maier 2014).

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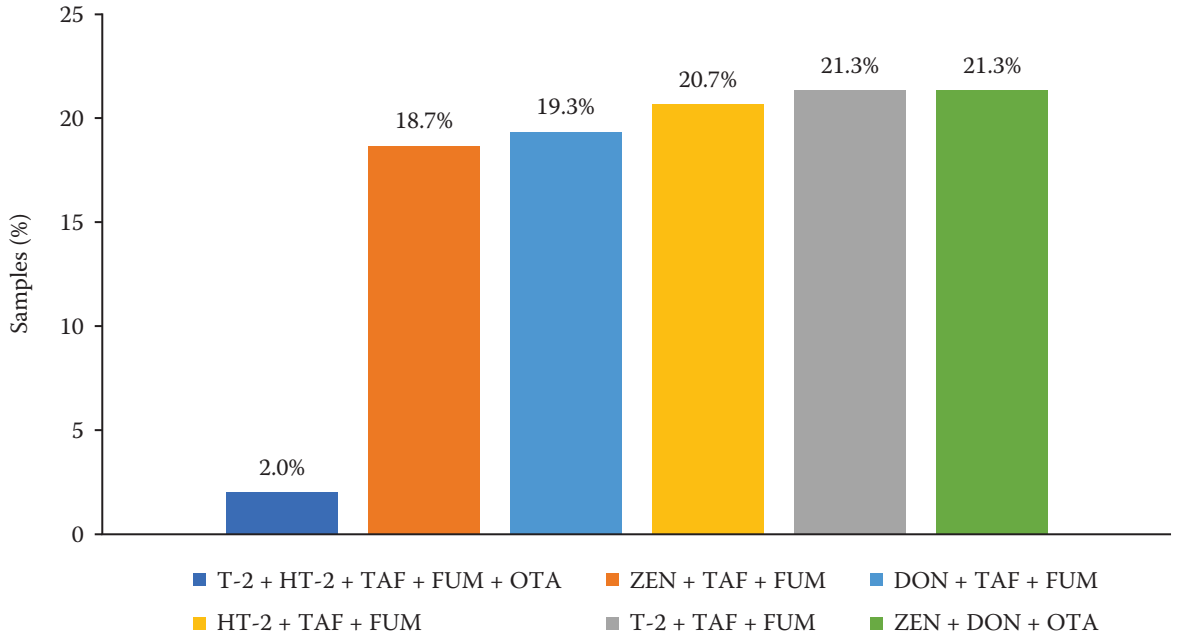

Figure 1. The co-occurrence of three or more mycotoxin types

TAF – total aflatoxins; FUM – total fumonisins; OTA – ochratoxin A; DON – deoxynivalenol; ZEN – zearalenone; HT-2 – HT-2 toxin; T-2 – T-2 toxin

CONCLUSION

Our research demonstrates the significance of increasing the frequency of sample collection and analysis for detecting mycotoxins in low-risk foods such as maize semolina chips. Improving maize storage conditions and processing processes is necessary as it poses a significant threat to public health. In addition, studies on the co-occurrence of the most common masked, modified, and other secondary fungal metabolites in maize and maize-produced foods are required. The most innovative technologies for controlling mycotoxin contamination in crops, such as biological control, and methods using the competitive exclusion of toxigenic strains by non-toxic ones, should be rolled out to general use. Other effective technologies, including irradiation, ozone fumigation, chemical and biological control agents, and improved packaging materials, should also be used to reduce postharvest aflatoxins contamination in agricultural products.

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