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Occurrence of *Alternaria* mycotoxins and aflatoxins contamination in vegetable oils by enzyme immunoassay study

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Abstract

Vegetable oils constitute a significant component of the human diet. The oilseeds utilized for their production are susceptible to contamination by mycotoxins (MTs) during cultivation and storage, particularly under suboptimal conditions. The extent and nature of fungal invasion leading to MT contamination also depends on the geographical origin of oilseed production. This study sought to investigate the prevalence of aflatoxins (AFs), alternariol (AOH), and tenuazonic acid (TEA) contamination in 18 types of edible vegetable oils using appropriate enzyme-linked immunosorbent assays (ELISAs). The oils examined ($n=102$) included common types such as sunflower, linseed, olive, mustard, sesame, hemp, and some others from the domestic market. The detection limits of the established assays were found to be consistent with the regulatory limits: 5, 10, and 100 µg/kg for AFs, AOH, and TEA, respectively. To ensure a satisfactory recovery of the analytes from the oil matrix, individual extraction solvents were necessary for AFB_1, AOH, and TEA. The recovery ranges of MTs from a wide range of common edible oils were found to be 68.8–99.8%, 63.9–114.1%, and 70.6–115.9%, respectively, with variation coeffecients of less than 19%. The ELISA detection limits of 0.003, 0.02, and 0.15 ng/mL provided high detectability of AFB_1 and AOH (73.5%), and TEA (66.6%) in the studied oils. However, their content above the maximum residue limits (MRLs) was observed only in 0, 4.9%, and 7.8% of the samples, respectively. The examination showed a notable decrease in the incidence and residual levels of AFs, AOH, and TEA in the refined sunflower oils compared to the unrefined oils. This study offers insights into the occurrence and MT contamination of vegetable oils within the Russian region and validates the efficacy of ELISA, in conjunction with optimized extraction protocols, for the routine analysis of a broad spectrum of oil types.

Keywords Aflatoxins, Alternariol, Tenuazonic acid, Vegetable oils, ELISA, Food safety

Introduction

Contamination of agricultural products with mycotoxins (MTs) presents a significant public health concern because of their diverse and severe toxic effects. Among the wide variety of mycotoxins produced by filamentous fungi such as *Aspergillus, Penicillium, Fusarium*, and *Alternaria*, certain compounds stand out for their proven severe toxicity. Aflatoxins (AFs), which are primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*, represent one of the most significant threats to food safety worldwide. Aflatoxin B_1 (AFB_1) is recognized

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as a potent naturally occurring carcinogen, causing significant damage to the liver and leading to severe health issues such as cell necrosis, hemorrhage, fibrosis, cirrhosis, and hepatocellular carcinoma [1].

Among the emerging MTs of particular concern are those produced by *Alternaria* species, which are increasingly detected in various food products [2]. Alternariol (AOH) has emerged as one of the most frequently occurring *Alternaria* toxins in oil-rich crops and their processed products [3]. Although the acute toxicity of AOH is considered low, increasing evidence highlights its potential to cause significant harm at relatively high concentrations. In vitro studies have demonstrated that AOH induces DNA damage, disrupts the cell cycle, promotes apoptosis, and interferes with immune cell function [4]. Furthermore, its ability to generate reactive oxygen species and interact with DNA topoisomerase raises concerns about long-term exposure [5].

Similarly, tenuazonic acid (TEA) has been found to inhibit the release of newly formed proteins from ribosomes. While TEA exhibits low in vitro toxicity, its in vivo effects are much more pronounced, including the development of hemorrhagic gastroenteropathy and organ damage in several animal models [6, 7].

The contamination of raw plant materials with MTs is a global problem that significantly impacts the safety of food and feed production, and causes serious economic damage. According to a large-scale study conducted in 100 countries, 88% of the analyzed raw material samples, including crops such as corn, wheat, and soybeans, were contaminated with at least one MT [8]. Particularly high levels of contamination have been recorded in regions with warm and humid climates, where MTs such as AFB_1 often exceed permissible limits [9–11]. However, fungal invasion and MT production in cereal crops can also be triggered by climate change or poor storage conditions. For example, in 2012, unusually hot and dry weather in Serbia led to significant contamination of maize with AFs, severely affecting its use for both food and oil production. In some samples, the levels of AFs significantly exceeded permissible limits, leading to losses in both domestic markets and exports [12]. The recent studies on sunflower from agricultural regions of Russia revealed a high level of seed contamination with MTs, especially AOH, largely due to unfavorable storage conditions, including self-heating, which contributes to MT accumulation [13, 14]. Therefore, the contamination of agricultural products with MTs is predominantly influenced by the geographical origin of specific raw materials and local climatic conditions.

Vegetable oils are included in the list of essential products and are important components of the daily human diet, despite the relatively low level of consumption (12 kg/year/person) [15]. It is therefore imperative that research and control of MT levels in oils be conducted in order to ensure food safety. However, the current level of knowledge about the contamination of edible vegetable oils with a wide variety of MTs, as well as the range of oils studied, is significantly limited. Thus, the Russian Federation's Regulation for safe residual MT content in vegetable oils has been established only for AFB_1 (5 μg/kg) [16]. The EU's recommended safe levels of AOH and TEA content are applicable only to sunflower oils, at 10 μg/kg and 100 μg/kg, respectively [17].

The analysis of MTs in vegetable oils typically involves chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS) [18]. These methods often require extensive sample preparation, including liquid–liquid extraction (LLE), solid–phase extraction (SPE), or precolumn derivatization, to increase sensitivity and specificity. Matrix-matched calibrations are also necessary to mitigate matrix effects [19]. Immunoanalytical methods such as enzyme-linked immunosorbent assay (ELISA) are generally simpler, higher throughput, more cost-effective, and allow rapid screening of multiple MTs 9simultaneously. Additionally, immunoassays often require easier sample preparation and can be adapted for onsite testing, making them highly suitable for routine monitoring in food safety applications. Nevertheless, the utilization of immunological methods in the examination of vegetable oils remains infrequent, seemingly due to the challenges associated with the analysis of oil matrixes.

For example, a wide variety of immunoassays developed for AFs have been reviewed in [20, 21], and the one presented in this study is comparable to the best in terms of sensitivity and limit of detection for AFs. However, very few studies have analyzed AFs in edible oils using immunoassays. The range of oil types analyzed and the global prevalence of AF contamination remain limited [22–24].

Reports on AOH immunoassays have focused on the analysis of this MT in fruits, fruit juices and wine [25–27]; corn, bran, flour and bread [28–30]; and oilseed-based animal feed [28]. The scope of TEA immunoassays in scientific literature is currently limited to sorghum grains and sorghum-based infant food [31], fruits and tomatoes [32], juices and beer [33]. The prevalence and extent of *Alternaria* toxin contamination in vegetable oils is largely unknown. In addition, the geographical features of vegetable oils from the Russian market and their susceptibility to contamination by Alternaria toxins have been practically unstudied.

The objective of the present study was to ascertain the prevalence of contamination of a wide variety of edible

vegetable oils produced and available in the domestic market with *Alternaria* toxins, AOH and TEA, as well as AFs, the most dangerous of the Aspergillus toxins. (Fig. 1).

This research encompasses the development of an immunoassay for the quantitative determination of MTs in vegetable oils. It also involves the optimization of the extraction procedure for each analyte from oils. Furthermore, it assesses the effect of oil refining on the degree of contamination and evaluates the prevalence of MT contamination in a wide range of oil types and compliance with food safety requirements.

Results and discussion

Development of ic-ELISAs for AFs, AOH, TEA and their analytical characteristics

Analytical systems based on indirect competitive ELISA (ic-ELISA) for MTs were constructed using previously prepared immunoreagents for AFs [34], AOH [28], and commercially available reagents for TEA. For the present study, a monoclonal antibody (mAb) with broad selectivity against AFs was chosen. It was able to recognize aflatoxins B_1, B_2, and G_1 as 100%, 89%, and 66%, respectively. The specificity of the rabbit anti-AOH polyclonal antibody was selective, with cross-reactivity to alternariol monomethyl ester less than 1%.

The typical standard curves for MTs in buffer and extractant media (Fig. 2A-C), along with the corresponding analytical characteristics of the developed ELISAs, are shown in Fig. 2D.

The parameters of the developed assays showed sufficient sensitivity to detect the analytes at their threshold concentrations, namely the maximum residue limits (MRLs) for AFB_1 and total AFs in edible oils established by the European Commission and Russian sanitary requirements of 2–5 µg/kg [16, 35], and the indicative levels of 10 and 100 µg/kg for AOH and TEA, respectively, as recommended by the European Commission for monitoring sunflower oils [17].

Examination of extraction efficiency

Antibodies, as biological molecules, are naturally designed to interact under physiological conditions, i.e., in an aqueous environment. Therefore, finding the most efficient way to transfer the analyte from its oil-dissolved state to the aqueous phase is crucial for successful analysis. The extraction efficiency of the MTs of interest is primarily related to their individual hydrophobic/hydrophilic properties. In this regard, the comparative effects of pure organic solvent methanol (MeOH) and assay buffer phosphate-buffered saline supplemented with 0.05% tween 20 (PBST, pH 7.2), as well as the effect of a 1:1 mixture of these extractants on analyte recovery were first elucidated.

For this, a panel of linseed oil samples ($n = 6$) was subjected to liquid–liquid extraction with the mentioned extractants according to a similar pretreatment procedure. To measure MT concentrations in oil extracts prepared and appropriately diluted with assay buffer, the corresponding standard curves in organic solvent and PBST were used (Fig. 2). The resulting extracts were analyzed with the developed ELISAs, and the data were compared. It was found that AFB_1 (Fig. 3A) and AOH (Fig. 3B) were more efficiently transfered into organic

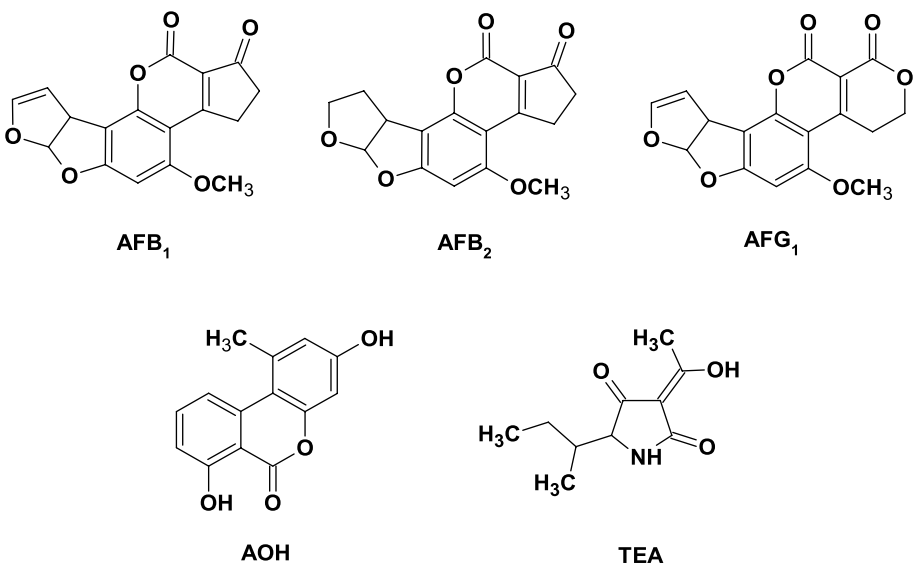
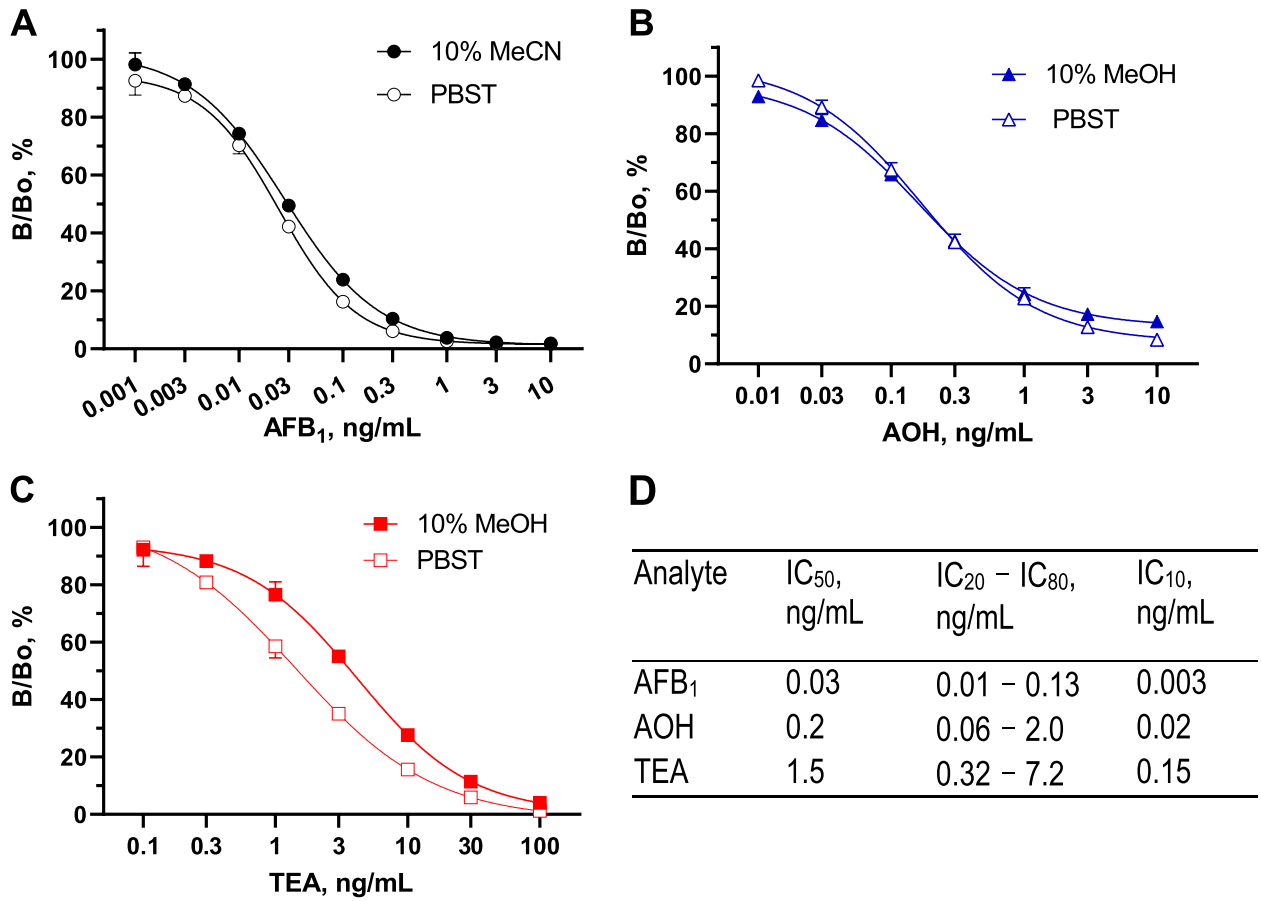


Fig. 1 Chemical structures of mycotoxins (MTs) determined via the developed enzyme-linked immunosorbent assays (ELISAs). AFB_1: Aflatoxin B_1; AFB_2: Aflatoxin B_2; AFG_1: Aflatoxin G_1; AOH: Alternariol; TEA: Tenuazonic acid



D

Analyte	IC_{50}, ng/mL	IC_{20} – IC_{80}, ng/mL	IC_{10}, ng/mL
AFB_1	0.03	0.01 – 0.13	0.003
AOH	0.2	0.06 – 2.0	0.02
TEA	1.5	0.32 – 7.2	0.15

Fig. 2 Standard curves of MTs AFB_1 (**A**), AOH (**B**), and TEA (**C**) and analytical characteristics (**D**) of the corresponding assays. Each symbol represents the average ($n = 3$) and standard deviation. Empty symbols are shown for standards in assay buffer, and filled symbol curves are calibrations in diluted extractant for determination in oil extract samples. MeCN: Acetonitrile; MeOH: Methanol; PBST: Phosphate-buffered saline with tween-20; IC_{10}: 10% inhibitory concentration; IC_{20}: 20% inhibitory concentration; IC_{50}: half maximal inhibitory concentration; IC_{80}: 80% inhibitory concentration

solvent, as their levels were significantly greater in the MeOH extracts than in PBST or MeOH/PBST mixture.

Under the same conditions, TEA prefers to enter the aqueous phase rather than the organic phase. Similar values obtained from the extraction of PBST and the MeOH/PBST mixture and negligible levels in the MeOH extracts indicate the hydrophilicity of TEA and confirm the suitability of PBST as a convenient extraction agent (Fig. 3C). Other reports have also confirmed that TEA has poor recovery rates when extracted with organic solvents. Even the extraction of TEA from various tomato products with aqueous acetonitrile (MeCN) resulted in only 17–73% recovery [36].

The initial comparative evaluation and selection between aqueous and organic solvents for MT extraction was further refined, showing that MeCN is the preferred solvent over MeOH for AF extraction, while MeOH remains the best solvent for AOH extraction. Thus, each of the analytes studied required its own individual extractant in order to be maximally extracted from the oil matrix: MeCN was the best for AFs extraction, MeOH was preferable for AOH, and PBST was more applicable for TEA.

Then, to identify the optimal extraction conditions, we tested different extraction durations (15 min, 1 day, and 1 week) and a modified extraction protocol using 4-fold solvent volume using AOH as a model analyte. These experiments were performed on two samples each of linseed and sunflower oil, with the oil/MeOH ratio maintained at 1:1 for the time-based experiments and adjusted to 1:4 for the increased solvent volume (Fig. 4).

Overall, the results suggest that neither extended extraction duration nor increased solvent volume had a significant effect on AOH recovery across most samples. The slight variations observed between different conditions were generally minimal and fell within the range of experimental error. Thus, these findings indicate that shorter extraction times (e.g., 15 min of intensive

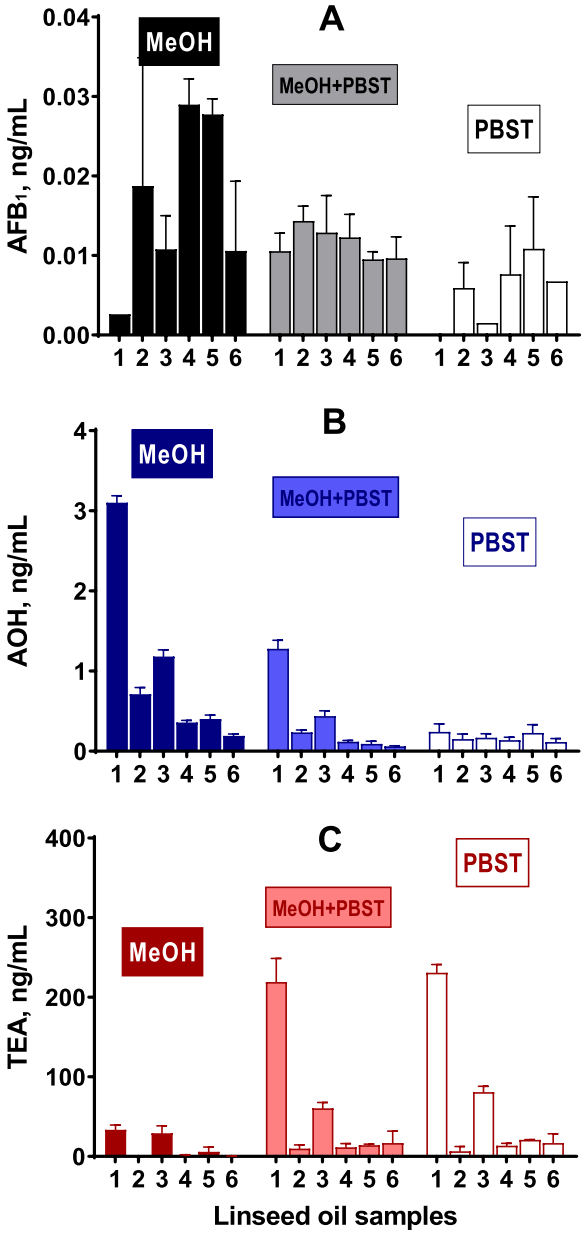


Fig. 3 Comparative extraction of AFB_1 (**A**), AOH (**B**), and TEA (**C**) from linseed oils ($n = 6$). Extractants used were methanol (MeOH), a methanol-PBST mixture (1:1), and PBST. MT levels are presented as averages ($n = 3$) with standard deviations

vortexing) and a standard solvent to sample volume ratio (1:1) are likely sufficient for routine AOH analysis, providing similar results comparable to those obtained with longer extraction times or increased solvent volumes. This streamlined protocol could thus offer time and resource efficiency without compromising extraction effectiveness.

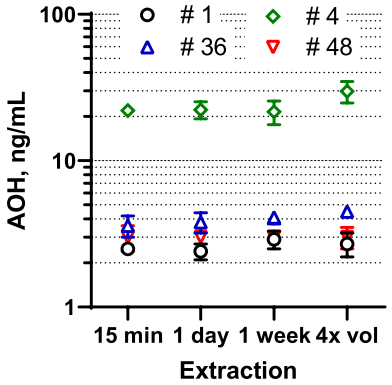


Fig. 4 Comparative efficiency of AOH extraction from linseed oils (#1 and #48) and sunflower oils (#4 and #36). The extraction duration was 15 min, 1 day and 1 week, with periodic shaking of the oil/MeOH (1:1) mixture and 1 day of extraction with the oil/MeOH (1:4) mixture

After optimization of the extraction protocols, recovery experiments were performed to confirm the efficiency of MT extraction for different oil matrices. Spikes were added to HPLC–MS/MS identified blank oil samples to obtain 1, 2, and 4 ng/mL AFB_1; 5, 10, and 15 ng/mL AOH; and 50, 100, and 200 ng/mL TEA (Table 1).

The recovery results presented show satisfactory extraction efficiencies achieved for AFB_1 (68.8–99.8%), AOH (63.9–114.1%), and TEA (70.6–115.9%) in all tested matrices, confirming the suitability of the established extraction procedure and the accuracy of the method for the determination of MTs at their threshold levels in a range of the most common edible oils with acceptable precision (coefficient of variation (CV) < 19%).

Analysis of mycotoxins in oil samples

The established extraction protocols for individual MTs were followed by appropriate dilution of the extract (ten-fold for AFB_1 and AOH; 50-fold for TEA) and subsequent analysis using the appropriate ELISA. The screening data from a panel of 102 collected oil samples provided insight into the prevalence and level of contamination of each oil type with the MTs of interest (Table 2).

Trace levels of AFs were detected in 47 (46.1%) samples out of 102 oils tested, none of which exceeded the critical threshold of 2 ng/mL (Table 2). Results obtained using HPLC–MS/MS also showed no AFB_1 contamination (< below limit of detection (LOD)) in dozens of oil samples selected for parallel confirmatory testing (Table S3). The low incidence of AF contamination is consistent with the results of other studies that have reported predominantly low or undetectable levels of AFB_1 in sunflower oils, even in southern regions. For example, 80.9% of sunflower oil samples in Tanzania had AFB_1 levels below the MRL of 2 ng/mL [37]. The same safety level was declared in the

Table 1 Recovery of MTs from oil samples via the developed ELISAs. CV: coefficient of variation; RC: recovery

Oil	AFB_1			AOH			TEA		
	Spiked, ng/mL	RC (%)	CV (%)	Spiked, ng/mL	RC (%)	CV (%)	Spiked, ng/mL	RC (%)	CV (%)
Sunflower	4	72.7	2.4	15	91.2	4.1	200	98.3	2.0
refined	2	91.9	1.5	10	70.0	6.9	100	105.8	12.3
	1	77.0	4.4	5	75.7	4.4	50	95.8	4.5
Sunflower	4	92.5	16.1	15	94.1	13.2	200	113.7	5,5
unrefined	2	91.9	4.2	10	110.4	11.7	100	115.9	11,1
	1	88.8	7.5	5	113.1	7.0	50	86.0	7,1
Linseed	4	98.8	10.6	15	76.9	9.7	200	81.0	5.6
	2	99.8	6.4	10	79.4	6.7	100	84.0	8.6
	1	86.1	6.9	5	70.6	10.5	50	101.9	10.0
Olive	4	81.2	15.5	15	90.1	6.6	200	97.9	8,8
	2	86.0	18.5	10	76.0	5.3	100	102.7	5,6
	1	99.0	12.9	5	114.1	5.3	50	73.6	8,6
Mustard	4	78.4	9.9	15	96.0	3,9	200	70.6	5.7
	2	99.4	12.6	10	101.6	3,3	100	91.1	8.3
	1	87.5	7.0	5	85.2	3,5	50	95.5	5.5
Sesame	4	68.8	2.1	15	63.9	18.1	200	89.1	16.3
	2	77.4	4.3	10	73.3	17.9	100	94.3	20.3
	1	104	8.5	5	91.6	12.1	50	94.9	15.7
Hemp	4	81.6	7.0	15	97.5	8.5	200	98.0	17.5
	2	94.0	6.1	10	87.9	9.6	100	99.3	5.5
	1	92.8	10.4	5	100.4	9.0	50	101.6	10.5

report from Nigeria for soya bean, groundnut, beniseed, palm kernel, melon and coconut oils [38], whereas 98% of Iranian sunflower oil samples were free of AFB_1 or within safe limits [39]. In addition, a meta-analysis highlighted that sunflower oil has one of the lowest average AFB_1 concentrations (2.64 µg/kg) among vegetable oils [40]. Thus, the studied oil samples collected in the Russian region (2021–2023) were not an exception to the above observations on AFs contamination of vegetable oils.

TEA was detected (> 7.5 ng/mL) by ELISA in more than half of the oil samples (61/102), with concentrations exceeding 100 ng/mL in only 8 samples (Table 2). Elevated levels of TEA (> 100 ng/mL) were found in sunflower oils (3/29), linseed oils (2/17 samples), but the highest incidence (3/5) and average residual level of TEA contamination (406.6 ng/mL) was found in hemp oils, which deserves further attention and study of this plant culture and oil type. The screening of Alternaria toxins by ELISA was verified by LC–MS/MS in parallel. Qualitative confirmation was obtained for positive and negative samples. However, quantitative results were inconsistent, probably because LC–MS/MS sample pretreatment for AOH and TEA analysis differed from ELISA pretreatment protocol, unlike AFB_1. MeOH and PBST extractions were chosen individually for the determination of AOH and TEA in ELISA, whereas a common extraction with MeCN was performed for the HPLC of all MTs. As shown above (Fig. 3), the type of solvent significantly affects the degree of analyte recovery and its quantification. Nevertheless, the revealed non-compliant samples were also identified by LC–MS/MS. (Table S3).

A similar incidence of contamination as with TEA was observed with another *Alternaria* toxin, AOH (Table 2), with 60.8% of the samples were positive (> 0.2 ng/mL). However, only two-thirds (42/62) of positive samples showed joint contamination with both TEA and AOH.

Only sunflower oil samples (5/29) were classified as non-compliant, exceeding 10 ng/mL level (Table 2). The average AOH concentration across all positive sunflower oil samples (25/29) was 5.4 ng/mL, while the average AOH content reported in a study analyzing sunflower oils ($n = 11$) in Germany was 27 µg/kg [41]. In another study from the European region [42], in which sunflower oil samples ($n = 16$) were analyzed, AOH was not detected in any of the refined or cold-pressed oils, whereas TEA was detected in a single sample at low concentrations (12.8 µg/kg). Similarly, an analysis of sunflower oils of Austrian-German origin ($n = 7$) showed that AOH was mostly undetectable, with TEA levels not exceeding 30 µg/kg [2]. At the same time, a study of a

Table 2 Examination of edible oils ($n = 102$) for AFs, AOH, and TEA residues by the ELISAs

Commodity (*n*)	AFB_1			AOH			TEA		
	Positive/non-compliant, n/n *	Range, ng/mL	Mean, ng/mL	Positive/non-compliant, n/n	Range, ng/mL	Mean, ng/mL	Positive/non-compliant, n/n	Range, ng/mL	Mean, ng/mL
Sunflower (29)	11/0	0.03–0.08	0.05	25/5	0.61–28.15	5.4	16/3	8.2–412.0	88.1
Linseed (17)	11/0	0.03–0.14	0.05	6/0	0.22–7.44	3.19	10/2	8.4–165.3	52.0
Olive (13)	6/0	0.04–0.08	0.06	10/0	0.77–7.86	2.59	9/0	19.1–69.0	33.8
Mustard (9)	8/0	0.03–0.07	0.05	2/0	0.33–0.81	0.57	8/0	2.8–47.0	27.9
Sesame (8)	2/0	0.08–0.08	0.08	7/0	0.82–9.06	2.48	3/0	20.2–32.3	26.0
Hemp (5)	3/0	0.05–0.11	0.08	4/0	1.36–5.36	3.33	5/3	10.6–1097	406.6
Pumpkinseed (3)	3/0	0.1–0.22	0.12	1/0	-	1.22	2/0	10.8–29.7	20.2
Pine nut (3)	1/0	-	0.03	2/0	0.33–3.26	1.80	1/0	-	27.9
Buckthorn (3)	0/0	-	0	2/0	1.88–3.34	2.61	2/0	24.4–33.1	28.8
Walnut (2)	1/0	-	0.03	1/0	-	4.9	0/0	-	0
Corn (2)	0/0	-	0	0/0	-	0	1/0	-	15.2
Castor (2)	0/0	-	0	1/0	-	0	2/0	11.1–12.8	12.0
Camelina (1)	0/0	-	0	0/0	-	0	0/0	-	0
Rice bran (1)	0/0	-	0	0/0	-	0	0/0	-	0
Rosehip (1)	1/0	-	0.03	1/0	-	1.38	0/0	-	0
Wheat germ (1)	0/0	-	0	0/0	-	0	1/0	-	34.6
Soybean (1)	0/0	-	0	0/0	-	0	0/0	-	0
Grapeseed (1)	0/0	-	0	0/0	-	0	1/0	-	24.7
Total (102), %/%	46.1/0			60.8/4.9			59.8/7.8		

* Positive samples were those > LOD, 0.03, 0.2, and 7.5 ng/mL (considering the dilution factor of extracts). Noncompliant samples were those > MRL, 2, 10, and 100 ng/mL for AFB_1, AOH, and TEA, respectively. Contamination level < LOD is indicated as zero concentration. Range and mean values are indicated for positive samples

wide range of oil types in India [3] found a much higher incidence (34%) of AOH contamination in 100 oil samples. The mentioned study showed the mustard oils were of the highest contamination level (mean 212 µg/kg) among other oil types, while the same value for the sunflower oils was 71.3 µg/kg. Mustard oils from our study ($n = 9$) showed no non-compliant AOH contamination.

Thus, the geographical origin of the raw materials used for oil production has a significant impact on the extent of fungal damage of oil crops, which in turn affects the contamination level of vegetable oils.

Impacts of oil refining on mycotoxin residue level

The MT levels in the oil samples analyzed can be affected by the processing methods. For example, the refining of vegetable oils may involve a number of steps, including extraction with organic solvents, treatment with acids and alkalis, high-temperature heating and hot steam, freezing, and filtration. The effect of such treatments on the residual MT content was assessed using sunflower oils as a model, since they were the only oils studied that were represented by refined ($n = 18$) and unrefined ($n = 11$) samples (Table S2). All other oil types, except corn ($n = 2$) and rice ($n = 1$), were cold-pressed oils.

The frequency of AF detection in unrefined oils was higher than in refined oils (55% vs. 28%) (Fig. 5), as well as the residual level (0.052 vs. 0.041 ng/mL), suggesting that the refining process can be as a way to reduce AF contamination.

A similar trend was observed regarding the effect of refining on the residual AOH content. The proportion of refined sunflower oil samples with non-compliant AOH content (> 10 ng/mL) was found to be only 5%, whereas the corresponding number for unrefined oils reached 36%. Additionally, the mean AOH concentration in the refined oils was found to be significantly lower (2.6 vs. 9.6 ng/mL).

The data also highlight the TEA removing as a result of refining (Fig. 5). Among the sunflower unrefined oils, 3 out of 11 (17%) samples exceeded 100 ng/mL, whereas no refined oils presented TEA levels above this threshold. Additionally, the mean TEA contamination level was found to be higher in unrefined oils (130 vs. 19.2 ng/mL). This aligns with previous findings, where cold-pressed oils consistently presented higher TEA levels than refined oils did [42]. Similarly, another study reported that organic, cold-pressed sunflower oils from Austria contained the highest levels of AOH (2.1–2.9 ng/g) and TEA (373–458 ng/g), whereas refined oils presented

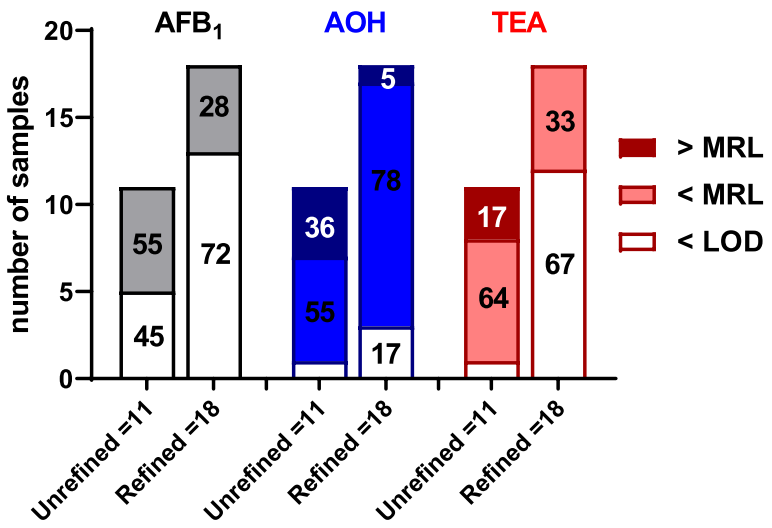


Fig. 5 Refining impact on MT residues in the sunflower oils. Blank columns present contamination level below assays' LOD 0.03 ng/mL (AFB_1), 0.2 ng/mL (AOH), and 7.5 ng/mL (TEA). Dark-colored columns indicate contamination level exceeding MRL: 2.0, 10, and 100 ng/mL for AFB_1, AOH, and TEA, respectively. The numbers in the columns indicate the percentages within the group

significantly lower toxin levels [43]. However, processing methods can have very different effects on MT residue levels. For example, Hickert et al. [44] reported high concentrations of AOH and TEA in sunflower seeds from South Africa, with TEA levels reaching up to 6260 ng/g. Interestingly, their study showed that seed shelling had varying effects on toxin concentrations, with TEA concentrations frequently elevated after shelling.

Conclusions

This study provides a comprehensive analysis of MT contamination in vegetable oils, with a focus on AFs, AOH, and TEA. Our findings confirmed that optimized extraction protocols enable the effective recovery of MTs from oil matrices, facilitating their accurate detection via ELISA. Among the 102 tested samples, AOH was found in 60.8% of the oils, with concentrations above the EU-recommended limit of 10 µg/kg observed only in sunflower oils (5/29). Similarly, TEA levels above the EU-recommended threshold of 100 µg/kg were detected in 7.8% samples of unrefined sunflower, linseed, and hemp oils. In contrast, AF contamination was minimal, with no samples exceeding the regulatory threshold of 5 µg/kg. The refining process was shown to reduce all MT levels, underscoring its importance for ensuring oil safety.

The results obtained shed light on the landscape of mycotoxin contamination of various vegetable oils produced in the Russian region, indicate the relative safety of these products, and also emphasize the need for regular monitoring of AOH and TEA content in raw materials used to produce vegetable oil, as well as control of MTs content in final products, especially in unrefined oils. The developed method provides a high throughput, reliable, and cost-effective approach for detecting multiple MTs in edible oils, supporting efforts to increase food safety standards and protect consumer health.

Materials and methods

Chemicals and reagents used

Aflatoxins B_1, B_2, G_1, AOH and TEA were gifts from Prof. Kononenko G.P. (Laboratory of Mycotoxicology, All-Russian Research Institute for Veterinary Sanitation, Hygiene and Ecology, Moscow, Russia). Anti-TEA mAb and BSA-TEA were obtained from Fapon (Guangdong, China). The MeOH and MeCN used were of analytical grade.

Indirect competitive enzyme-linked immunosorbent assay (icELISA)

An indirect competitive ELISA method was used to detect MTs produced by *Aspergillus* and *Alternaria*. Conjugated MTs, namely, GEL-AFB_1, GEL-AOH, and BSA-TEA, were coated on 96-well Costar plates in 100 µL solutions (0.05–1.5 µg/mL) in 0.05 M carbonate buffer (pH 9.6) overnight at 4 °C. The plates were washed three times with phosphate-buffered saline supplemented with 0.05% tween 20 (PBST, pH 7.2) and then filled with 100 µL of MT standards (0, 0.01–1000 ng/mL) or samples and 100 µL of the appropriate specific antibody in 1% BSA-PBST. The reaction mixtures were incubated for 1 h at 25 °C in a plate thermoshaker chamber 3ST- 3 L (ELMI Ltd. Riga, Latvia) to establish an equilibrium interaction between the competing free analyte and the coating conjugate for binding to the antibody. The excess unbound reagents were removed from the wells by washing. The

immune complexes formed with immobilized antigens were detected via anti-species IgG peroxidase conjugates (GAR-HRP or RAM-HRP, Imtek, Moscow, Russia). After 1 h of incubation at 37 °C and washing, 100 µL of TMB-substrate mixture was added to each well to detect the amount of bound enzyme conjugate. Color product development was terminated 30 min later by the addition of 100 µL of 2 M sulfuric acid. The absorbance was measured at 450 nm via a LisaScan reader (Erba Manheim, Karásek, Czech Republic). The average signal values in wells with zero (B_0) and other (B) concentrations of the MT standard served to construct a calibration plot as the MT concentration versus the relative binding of antibodies (B/B_0). The MT concentrations that inhibited antibody binding by 10% (IC_{10}), 50% (IC_{50}) and 20–80% (IC_{20}-IC_{80}) have been qualified according to common practices as the detection limit, assay sensitivity and operating range values, respectively [45].

Accuracy and precision

Recoveries and coefficients of variation (CVs) were calculated to evaluate the accuracy and precision. Several blank oils of different types, verified to be free of MT residues by HPLC–MS/MS, were spiked with AFB_1 at concentrations close to the MRL concentrations (1, 2, and 4 ng/mL). The same oil types were spiked with AOH (5, 10, and 15 ng/mL) or with TEA (50, 100, and 200 ng/mL). The fortified samples were stirred vigorously for 15 min and then subjected to the appropriate extraction procedure and analyzed via the developed ELISAs. Recovery rates were estimated as the percentage ratio between the measured and spiked concentrations.

Sample pretreatment

All edible oil samples ($n = 102$) were purchased from a domestic retail chain in 2021–2023. Sample collection was guided by the maximum diversity of manufacturers, brands or production batches so that all analyzed samples were individual and unique. The groups of edible oils studied included: sunflower ($n = 29$), linseed ($n = 17$), olive ($n = 13$), mustard ($n = 9$), sesame ($n = 8$), and hemp ($n = 5$); three samples each of pumpkin seed, pine nut, and buckthorn oils; two samples each of walnut, corn, and castor oils; and one sample each of camelina, rice bran, rose hip, wheat germ, soybean, and grapeseed oils.

An equal volume of extractant was added to each oil aliquot in tubes and vortexed thoroughly for 15 min, followed by centrifugation at 6800 ×g for 5 min. Different solvents, namely, MeCN, MeOH, and PBST, were compared in terms of extraction efficiency. The extractant layer separated from the oil after centrifugation was carefully aspirated, diluted 10-, 10-, and 50-fold with PBST, and tested by ELISA to quantify AFB_1, AOH, and TEA, respectively.

HPLC–MS/MS procedure

The basic HPLC–MS/MS procedure did not differ from that described in a recent report [46] and is described in detail with appropriate sample pretreatment methods in the Supplementary Information.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44280-025-00075-1.

Supplementary Material 1.

Authors'contributions

M.A.B. and A.O.M. designed the experiments; M.A.B., A.G.M. and F.D.S. wrote the manuscript; I.A.G., A.G.M., F.D.S. and A.O.M. performed the experiments; I.A.G., A.G.M. and F.D.S. analyzed the data; M.A.B. and A.G.M. provided discussion and revised the manuscript. All the authors have read and approved the final manuscript.

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Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests. Author Maksim A. Burkin is a member of the Editorial Board for *One Health Advances*. He was not involved in the journal's review and decisions related to this manuscript.

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References

1. Ferreira RG, Cardoso MV, De Souza Furtado KM, Espíndola KMM, Amorim RP, Monteiro MC. Epigenetic alterations caused by aflatoxin b1: a public health risk in the induction of hepatocellular carcinoma. Transl Res. 2019;204:51–71.
2. Puntscher H, Kütt ML, Skrinjar P, Mikula H, Podlech J, Fröhlich J, et al. Tracking emerging mycotoxins in food: development of an LC-MS/MS method for free and modified *Alternaria* toxins. Anal Bioanal Chem. 2018;410(18):4481–94.
3. Bansal M, Saifi IJ, Dev I, Sonkar AK, Dixit S, Singh SP, et al. Occurrence of Alternariol and Alternariol monomethyl ether in edible oils: their thermal stability and intake assessment in state of Uttar Pradesh. India J Food Sci. 2021;86(3):1124–31.
4. Solhaug A, Eriksen GS, Holme JA. Mechanisms of action and toxicity of the mycotoxin alternariol: a review. Basic Clin Pharma Tox. 2016;119(6):533–9.

5. den Hollander D, Holvoet C, Demeyere K, De Zutter N, Audenaert K, Meyer E, et al. Cytotoxic effects of alternariol, alternariol monomethyl-ether, and tenuazonic acid and their relevant combined mixtures on human enterocytes and hepatocytes. Front Microbiol. 2022;22(13): 849243.
6. Chen A, Mao X, Sun Q, Wei Z, Li J, You Y, et al. *Alternaria* mycotoxins: an overview of toxicity, metabolism, and analysis in food. J Agric Food Chem. 2021;69(28):7817–30.
7. Fraeyman S, Croubels S, Devreese M, Antonissen G. Emerging *Fusarium* and *Alternaria* mycotoxins: occurrence, toxicity and toxicokinetics. Toxins. 2017;9(7):228.
8. Gruber-Dorninger C, Jenkins T, Schatzmayr G. Global mycotoxin occurrence in feed: a ten-year survey. Toxins. 2019;11(7):375.
9. Gruber-Dorninger C, Novak B, Nagl V, Berthiller F. Emerging mycotoxins: beyond traditionally determined food contaminants. J Agric Food Chem. 2017;65(33):7052–70.
10. Kovalsky P, Kos G, Nährer K, Schwab C, Jenkins T, Schatzmayr G, et al. Co-occurrence of regulated, masked and emerging mycotoxins and secondary metabolites in finished feed and maize—an extensive survey. Toxins. 2016;8(12):363.
11. Qi N, Yu H, Yang C, Gong X, Liu Y, Zhu Y. Aflatoxin B_1 in peanut oil from Western Guangdong, China, during 2016–2017. Food Addit Contam Part B. 2019;12:45–51.
12. Luo S, Du H, Kebede H, Liu Y, Xing F. Contamination status of major mycotoxins in agricultural product and food stuff in Europe. Food Control. 2021;127:108120.
13. Burkin AA, Ustyuzhanina MI, Zotova EV, Kononenko GP. Reasons of contamination of production lots of sunflower (*Helianthus Annuus* L.) seeds by mycotoxins. Agric Biol. 2018;53(5):969–76. https://doi.org/10.15389/agrobiology.2018.5.969eng
14. Kononenko GP, Ustyuzhanina MI, Burkin AA. The problem of safe sunflower (*Helianthus Annuus* L.) use for food and fodder purposes (review). Agric Biol. 2018;53(3):485–98.
15. Ministry of Healthcare of the Russian Federation, No. 614 of 19 August 2016. Recommendations on rational norms for the consumption of food products that meet modern requirements for a healthy diet. https://www.consultant.ru/document/cons_doc_LAW_204200/. Accessed 10 Dec 2024.
16. SanPiN. Hygienic requirements in respect of the safety and nutritional value of foodstuffs, SanPiN 2.3.2.1078–01, as amended. 2. Chapter 1. Requirements for the safety and nutrition of foods of the unified sanitary-epidemiological and hygiene requirements of the commission of the customs union of Russia, Belarus and Kazakhstan. https://docs.cntd.ru/document/902249109. Accessed 10 Dec 2024.
17. European Commission. Commission recommendation (EU) 2022/553 of 5 April 2022 on monitoring the presence of *Alternaria* toxins in food. Off J Eur Union. 2022;107(90):2022.
18. Zhou J, Yao SS, Wang JM, Chen XH, Qin C, Jin MC, et al. Multiple mycotoxins in commonly used edible oils: occurrence and evaluation of potential health risks. Food Chem. 2023;426:136629.
19. Abdolmaleki K, Khedri S, Alizadeh L, Javanmardi F, Oliveira CAF, Mousavi KA. The mycotoxins in edible oils: an overview of prevalence, concentration, toxicity, detection and decontamination techniques. Trends Food Sci Technol. 2021;115:500–11.
20. Li P, Zhang Q, Zhang W. Immunoassays for aflatoxins. TrAC Trends Anal Chem. 2009;28(9):1115–26.
21. Yan C, Wang Q, Yang Q, Wu W. Recent advances in aflatoxins detection based on nanomaterials. Nanomaterials. 2020;10(9):1626.
22. Sun Q, Zhu Z, Deng QM, Liu JM, Shi GQ. A "green" method to detect aflatoxin B_1 residue in plant oil based on a colloidal gold immunochromatographic assay. Anal Methods. 2016;8(3):564–9.
23. Zhang D, Li P, Yang Y, Zhang Q, Zhang W, Xiao Z, et al. A high selective immunochromatographic assay for rapid detection of aflatoxin B_1. Talanta. 2011;85(1):736–42.
24. Zhang Y, Li M, Cui Y, Hong X, Du D. Using of tyramine signal amplification to improve the sensitivity of ELISA for aflatoxin B_1 in edible oil samples. Food Anal Methods. 2018;11(9):2553–60.
25. Ackermann Y, Curtui V, Dietrich R, Gross M, Latif H, Märtlbauer E, et al. Widespread occurrence of low levels of alternariol in apple and tomato products, as determined by comparative immunochemical assessment using monoclonal and polyclonal antibodies. J Agric Food Chem. 2011;59(12):6360–8.
26. Addante-Moya LG, Suay-Fuentes D, Abad-Somovilla A, Agulló C, Abad-Fuentes A, Mercader JV. A rapid monoclonal antibody-based direct immunoassay and a lateral-flow device for on-site sensitive analysis of the mycotoxin alternariol. Microchem J. 2024;199:109892.
27. Man Y, Liang G, Jia F, Li A, Fu H, Wang M, et al. Development of an immunochromatographic strip test for the rapid detection of alternariol monomethyl ether in fruit. Toxins. 2017;9(5):152.
28. Burkin AA, Kononenko GP. Enzyme immunassay of alternariol for the assessment of risk of agricultural products contamination. Appl Biochem Microbiol. 2011;47(1):72–6.
29. Singh G, Velasquez L, Brady B, Koerner T, Huet AC, Delahaut P. Development of an indirect competitive ELISA for analysis of alternariol in bread and bran samples. Food Anal Methods. 2018;11(5):1444–50.
30. Wang J, Peng T, Zhang X, Yao K, Ke Y, Shao B, et al. A novel hapten and monoclonal antibody-based indirect competitive ELISA for simultaneous analysis of alternariol and alternariol monomethyl ether in wheat. Food Control. 2018;94:65–70.
31. Gross M, Asam S, Rychlik M. Evaluation of an enzyme immunoassay for the detection of the mycotoxin tenuazonic acid in sorghum grains and sorghum-based infant food. Mycotoxin Res. 2017;33(1):75–8.
32. Gross M, Curtui V, Ackermann Y, Latif H, Usleber E. Enzyme immunoassay for tenuazonic acid in apple and tomato products. J Agric Food Chem. 2011;59(23):12317–22.
33. Liang YF, Zhou XW, Wang F, Shen YD, Xiao ZL, Zhang SW, et al. Development of a monoclonal antibody-based ELISA for the detection of alternaria mycotoxin tenuazonic acid in food samples. Food Anal Methods. 2020;13(8):1594–602.
34. Burkin MA, Yakovleva IV, Sviridov VV, Burkin AA, Kononenko GP, Soboleva NA. Comparative immunochemical characterization of polyclonal and monoclonal antibodies to aflatoxin B_1. Appl Biochem Microbiol. 2000;36(5):496–501.
35. European Commission. Commission regulation (EU) No 165/2010 of 26 February 2010 amending regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. Off J Eur Union. 2010;50(8):2010.
36. Ji X, Deng T, Xiao Y, Jin C, Lyu W, Wu Z, et al. Emerging *Alternaria* and *Fusarium* mycotoxins in tomatoes and derived tomato products from the China market: occurrence, methods of determination, and risk evaluation. Food Control. 2023;145:109464.
37. Mohammed S, Munissi JJE, Nyandoro SS. Aflatoxins in sunflower seeds and unrefined sunflower oils from Singida, Tanzania. Food Addit Contam: Part B. 2018;11(3):161–6.
38. Malu SP, Donatus RB, Ugye JT, Imarenezor EPK, Leubem A. Determination of Aflaxtoxin in some edible oils obtained from Makurdi Metropolis, North Central Nigeria. Am J Chem Appl. 2017;4:36–40.
39. Nabizadeh S, Shariatifar N, Shokoohi E, Shoeibi S, Gavahian M, Fakhri Y, et al. Prevalence and probabilistic health risk assessment of aflatoxins B_1, B_2, G_1, and G_2 in Iranian edible oils. Environ Sci Pollut Res. 2018;25(35):35562–70.
40. Einolghozati M, Talebi-Ghane E, Ranjbar A, Mehri F. Concentration of aflatoxins in edible vegetable oils: a systematic meta-analysis review. Eur Food Res Technol. 2021;247(12):2887–97.
41. Hickert S, Bergmann M, Ersen S, Cramer B, Humpf HU. Survey of *Alternaria* toxin contamination in food from the German market, using a rapid HPLC-MS/MS approach. Mycotoxin Res. 2016;32(1):7–18.
42. Tölgyesi Á, Kozma L, Sharma VK. Determination of *Alternaria* toxins in sunflower oil by liquid chromatography isotope dilution tandem mass spectrometry. Molecules. 2020;25(7):1685.
43. Puntscher H, Cobankovic I, Marko D, Warth B. Quantitation of free and modified *Alternaria* mycotoxins in European food products by LC-MS/MS. Food Control. 2019;102:157–65.

44. Hickert S, Hermes L, Marques LMM, Focke C, Cramer B, Lopes NP, et al. *Alternaria* toxins in South African sunflower seeds: cooperative study. Mycotoxin Res. 2017;33(4):309–21.
45. Burkin MA, Galvidis IA. Simultaneous immunodetection of ionophore antibiotics, salinomycin and narasin, in poultry products and milk. Anal Methods. 2021;13(13):1550–8.
46. Burkin MA, Tevyashova AN, Bychkova EN, Melekhin AO, Galvidis IA. Immunotechniques for the group determination of macrolide antibiotics traces in the environment using a volume-mediated sensitivity enhancement strategy. Biosensors. 2023;13(10):921.

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