Aflatoxin B1 in the Iranian pistachio nut and decontamination methods: A systematic review

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Abstract

This study aimed to summarize the occurrence of aflatoxin B_1 (AFB_1) in the various cultivars of the Persian pistachio nut and the decontamination techniques, with the aid of a systematic review and meta-analysis. In this regard, all published studies up to November 2019 among international and national databases were screened, resulting in 23 articles. According to the findings, the main aflatoxin identified in the pistachio nut was AFB_1, with AFB_1 concentrations in ~28% of the studies being ≤ 5 µg/kg, in ~35% of the studies being ≤ 10 µg/kg, and in ~37% of the studies being ≥ 10 µg/kg. Generally, in most studies (~72%), AFB_1 content in pistachio cultivars was higher than the acceptable level proposed by the Iranian National Standard. Although several physical and chemical techniques for AFB_1 reduction have been introduced, most of them are not safe and/or suitable for human consumption.

Keywords: Pistachio nut; aflatoxin $B_{1;}$ prevalence; systematic review; decontamination

Introduction

Pistachio (*Pistacia vera* L.), an important plant of the *Anacardiaceae* family, is one of the main Iranian agricultural products (Kola *et al*., 2018; Mokhtarian *et al*., 2017; Tavakolipour and Mokhtarian, 2014; Tavakolipour *et al*., 2017). In 2016, the United States, Iran, and Turkey were the leading pistachio producers globally, reaching 1,335,480, 1,034,999, and 558,303 metric tons, respectively (FAO, 2016). The pistachio nut is consumed in several countries, either as a snack or as an ingredient in food preparation (kernel or powder). A rich composition, including 25% protein (usually essential amino acids), 10% dietary fiber, 55% oil (~80% unsaturated fatty acids), and 16% carbohydrates, provides a high nutritional value for many pistachio-based, traditional Iranian deserts, including Baghlava, Gaz and Ghotab (Mokhtarian *et al.*, 2016; Tavakolipour and Mokhtarian, 2016; Tavakolipour *et al.*, 2017). Although pistachio plays an essential role as a major food item in Iran, more than half of the annual Iranian production is exported, thus requiring appropriate production systems for the exported nuts' quality assurance.

Aspergillus parasiticus, A. nomius, and *A. flavus* are important toxigenic fungi that can infect various nuts,

such as pistachio, and contaminate them with aflatoxin G_1 (AFG_1), B_1 (AFB_1), G_2 (AFG_2), and B_2 (AFB_2) (Abdallah *et al*., 2020; Aydin and Ulvi, 2019; Ehrlich *et al*., 2007). AFB_1 is a genotoxic carcinogen (Hussein and Brasel, 2001), which leads to toxic effects on the kidney, liver, hematopoietic stem cells, immune system, and the fetal and reproductive systems (Heshmati *et al*., 2017; Khaneghah *et al*., 2018, 2019; Nabizadeh *et al*., 2018). In this regard, the Iranian Scientific Committee for Food (SCF) has reported that AFB_1 is a major factor in cancer, especially for the liver, even at very low amounts (Bensassi *et al*., 2010). According to Iran's national standard, the maximum tolerated levels for AFB_1 and the total aflatoxin (TAFT) in pistachio are 5 and 15 µg/kg, respectively (ISIRI, 2002). However, the European Commission standardized the aflatoxin (AFT) content of pistachio as <10 µg/kg (European Commission, 2010). The prevalence of AFT in the pistachio nuts has been reported previously. In 2015, the Rapid Alert System for Food and Feed (RASFF) reported a total of 475 alerts for mycotoxins, of which 89% were associated with AFT, mostly from nuts (81%), mainly pistachio nuts, pumpkin seeds, and dried mix nuts (Pigłowski, 2019; RASFF, 2016). Georgiadou *et al.* (2012) investigated the AFT contamination in the pistachio nuts. In order to determine the rate of AFT contamination in the pistachio nuts, four orchards were chosen, and 20 samples (total of 80 samples) were selected from each orchard. It should be noted that AFT control of food commodities requires well-developed analytical methods (Mousavi Khaneghah *et al*., 2018). The combination and structure of food products, identification, and mycotoxins detection is a challenge (Campagnollo *et al*., 2016; Heshmati *et al*., 2017). Therefore, analytical methods for precise quantification of mycotoxins in the pistachio nuts have been developed, aiming to provide high sensitivity, easy-to-operate steps, rapidness, and low-cost sample preparation procedures (Rahmani *et al*., 2018).

In recent years, several technological approaches have been introduced to reduce the growth of fungi or for the removal of AFT from pistachios, including the application of extracts and essential oils from medicinal plants (Ijabadeniyi *et al*., 2020; Khorasani *et al*., 2017); ozone treatment (O_3) (Chen *et al*., 2014); the use of chemical compounds, such as methyl bromide (CH_3Br), hydrogen peroxide (H_2O_2), sodium bisulfite ($NaHSO_3$), and ammonia; the use of adsorbent compounds, such as active charcoal, zeolite, sodium bentonite, hydrated sodium calcium aluminosilicate (HSCAS); gamma radiation (Serra *et al*., 2018); and biological methods, such as mannan compounds from the cell wall of *Saccharomyces cerevisiae* and lactic acid bacteria (Abdallah *et al*., 2018; Abdolshahi *et al*., 2018). Examples of the application of biological methods include the report by Abdolshahi *et al.* (2018), who studied the interacting capacity of *S. cerevisiae* mannoprotein (mannan) with AFT in pistachio by soaking the kernels in mannan solutions at 25 and 50 mg/mL. Siahmoshteh *et al.* (2017) investigated the efficacy of *B. amyloliquefaciens* and *B. subtilis* in controlling AFT production and *Aspergillus parasiticus* growth. The authors considered the *Bacillus* species as good biocontrol agents to reduce toxigenic fungal material growth and the subsequent AFT decontamination of pistachio. Rastegar *et al.* (2017) investigated the removal of AFB_1 by roasting contaminated pistachio nuts mixed with lemon juice and/or citric acid.

It can be stated that, due to the economic, nutritional, and health aspects of the Iranian pistachio nut (Mokhtarian, 2015; Ros, 2010), along with the high susceptibility of this product to fungal growth (especially to *Aspergillus* spices), there is a need for strategies to reduce AFT contamination at different stages of harvest, postharvest, and during the processing of this product. Therefore, this study aimed to review the data from all available studies on the prevalence of AFB_1 in different Iranian pistachio cultivars and recommend effective ways to combat this severe challenge.

Search Strategy

Keywords and screened databases

In order to study the prevalence of AFB_1 in the different Iranian pistachio cultivars, all published studies up to December of 2018 were screened using "Scopus," "PubMed," and "SID" databases using the keywords, "Pistachio nut," "Pistachio kernel," "Aflatoxin contamination," "*Aspergillus flavus*," "Anti-aflatoxigenic," "Anti-mycotoxigenic," "Iranian," and "Persian." Also, all references in the retrieved studies were reviewed to prevent any study from being missed out. It should be noted that all studies that examined the analytical methods of AFT detection in pistachios were excluded from the research.

Data extraction

Considering that the pistachio is one of Iran's nonoil and strategic products, in this study, only the research carried out by Iranian researchers was addressed. From each study, the required information, including author name (researcher), publication year, analytical technique, pistachio cultivar, an average of the reported AFB_1, number of samples, the ratio of the measured AFT to the maximum tolerated level of AFB_1 following the Iranian national standard AFB_{1R}/AFB_{1S}, and the method applied to reduce AFTs in pistachio were obtained.

Main Findings

Study characteristics

In the initial evaluation of the 1582 articles collected, 674 were excluded because of duplication (repeated articles) and irrelevant data descriptions. A total of 908 articles extracted as the primary articles based on abstracts and titles were excluded because 835 articles reported on other toxins and 73 articles did not describe the analytical method used. Only those researches reporting the AFT contamination with the particular analytical method used and studies on decontamination techniques in pistachio nuts were evaluated. At the end of the data extraction procedure, 23 articles published before 30 December 2018 were selected and evaluated in our study (Tables 1, 2, and Figure 1).

Table 1. Published researches related to the prevalence of AFB_1 contamination in the pistachio nuts produced in Iran.

Pistachio cultivar	Average of AFB_1 reported in research (µg/kg)	Number of studied samples (N)	Ratio $\frac{AFB_{1R}}{AFB_{1S}}$ ** ***	Identification method	Ref.
O'hadi	285.5	10	~57	HPLC	Mehrnezhad and Panahi, 2005
	1485	14	~297		
O'hadi	6.25	–	1.25	TLC	Pak-kish and Rahemi, 2005
Akbari	10	–	2	HPLC	Rahaie *et al*., 2010b
Akbari	5.63	–	1.126	HPLC	Tavakolipour *et al*., 2012
Fandoghi (Early split)	–	7.93	~1.59	ELISA	Dargahi *et al*., 2014
Fandoghi (Whole)	1.59	–	~0.32	ELISA	Dargahi *et al*., 2014
Unknown	~0.681	72	~0.136	HPLC	Shakeri and Fazaeli, 2016
Kermani	5.9	3356	1.18	HPLC	Cheraghali *et al*., 2007
Unknown	8.12	–	~1.63	HPLC	Aghamohammadi *et al*., 2007
Kermani	~311.6	3181	~62.32	HPLC	Dini *et al*., 2013
Ahmad Aghaei (Damghan)	2.077	600	~0.42	HPLC	Taghizadeh *et al*., 2018
Akbari (Damghan)	1.948	600	0.3896		
Kalle-Ghuchi (Damghan)	1.627	600	0.3254		
O'hadi (Damghan)	<0.066	600	0.0132		
Badami (Damghan)	<0.066	600	0.0132		
Ahmad Aghaei (Feyz Abad)	3.863	600	0.7726		
Akbari (Feyz Abad)	3.081	600	0.6162		
Kalle-Ghuchi (Feyz Abad)	2.143	600	0.4286		
O'hadi (Feyz Abad)	0.2	600	0.04		
Badami (Feyz Abad)	0.2	600	0.04		
Ahmad Aghaei (Rafsanjan)	4.33	600	0.866		
Akbari (Rafsanjan)	4.08	600	0.816		
Kalle-Ghuchi (Rafsanjan)	3.453	600	0.6906		
O'hadi (Rafsanjan)	0.2	600	0.04		
Badami (Rafsanjan)	0.2	600	0.04		
Ahmad Aghaei (Sarakhs)	0.961	600	0.1922		
Akbari (Sarakhs)	0.487	600	0.0974		
Kalle-Ghuchi (Sarakhs)	0.233	600	0.0466		
O'hadi (Sarakhs)	<0.066	600	0.0132		
Badami (Sarakhs)	<0.066	600	0.0132		
Unknown	22.02****	43	~4.4	ELISA	Ostadrahimi *et al*., 2014

*Maximum tolerated level of AFB1 in accordance with the Iranian National Standard (No. 5925, 1380), 5 µg/kg (ISIRI, 2002).
**AFB1R: Obtained AFB1 content in accordance with the research.
***AFB1S: The AFB1 content in accordance with the Iranian National Standard (1380).
****In this research, the noted value is for TAFT.
AFBB1, aflatoxin B_1; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; ELISA, enzyme-linked immunosorbent assay.

Table 2. The published applied researches related to the decontamination methods of AFB_1 in the pistachio nuts produced in Iran.

Pistachio cultivar	Average of AFB1 (µg/kg)		Aflatoxin decontamination method	Amount of toxin removal (%)	Ref.
	Before treatment	After treatment			
O'hadi	6.25	1.2	25 mg/l Gibberellic acid	~81	Pak-kish and Rahemi, 2005
Ahmad Aghaei	~2.75	~0.5	Phenolics of pistachio green hull	~81	Afshari *et al*., 2009
Akbari	10	6	Inoculation of pistachio by *Saccharomyces cerevisiae* yeast	40	Rahaie *et al*., 2010a
Akbari	16.467	5.644	Extract of Shirazi thyme	~66	Tavakolipour *et al*., 2010
Shahpasand	32,300	31,171	Testa (Pistachio kernel coat)	~3.49	Mohammadi Moghadam *et al*., 2011
Abasali	32,307	30,754		~4.81	
O'hadi	32,810	29,823		~9.11	
Ahmad Aghaei	30,171	29,429		~2.46	
Akbari	29,940	26,880		~10.22	
Kale-Bozi	27,439	26,410		~3.75	
Kalle-Ghuchi	30,433	26,218		~13.85	
FAS-13-73	29,012	25,393		~12.47	
Fakhri	30,762	24,811		~19.35	
Kal-e-Khandan	27,912	21,728		~22.15	
Akbari	16.467	5.644	Extract of the Shirazi thyme	~66	Tavakolipour *et al*., 2012
Unknown	1700.63	610.32	*Trichoderma* spp. T_1 extracellular extract	~64.11	Chegini, Behbodi *et al*., 2013
		265.29	*Trichoderma* spp. T_3 extracellular extract	~84.4	
		376.03	*Trichoderma* spp. T_4 extracellular extract	~77.88	
		825.75	*Trichoderma* spp. T_{17} extracellular extract	~51.44	
O'hadi	–	–	4 ppm soluble ozone in water	32.7	Bashiri *et al*., 2013
	–	–	8 ppm soluble ozone in water	47.9	
Unknown	–	–	Radiation at 1 kGy	38.84	Haji Mohammadi *et al*., 2016
	–		Radiation at 3 kGy	48.79	
	–		Radiation at 5 kGy	53.50	
	–		Radiation at 7 kGy	77.17	
Unknown (Pistachio kernel)	44		Roasted at 150°C (30 min)	66	Yazdanpanah *et al*., 2005
	91			64	
	213	–		24	
Unknown (Whole pistachio)	144	–	Roasted at 150°C (30 min)	63	
	253	–		19	
Unknown	~2500	~450	Inoculation of pistachio by *Bacillus subtilis* (UTBSP1) for 96 h at 30°C	82	Farzaneh *et al*., 2012
Ahmad Aghaei	–	–	Inoculation of pistachio by *Bacillus amyloliquefaciens* (UTBSP1) for 5 days at 30°C	23.9	Siahmoshteh *et al*., 2017
	–		Inoculation of pistachio by *Bacillus amyloliquefaciens* (UTBSP1) for 8 days at 30°C	54.9	
	–		Inoculation of pistachio by *Bacillus subtilis* for 5 days at 30°C	41.1	
	–		Inoculation of pistachio by *Bacillus subtilis* for 8 days at 30°C	52.5	

(*continues*)

Table 2. Continued

Pistachio cultivar	Average of AFB1 (µg/kg)		Aflatoxin decontamination method	Amount of toxin removal (%)	Ref.
	Before treatment	After treatment			
Unknown	268	–	Roasting of pistachio at 120°C for 60 min by incorporation of 30 mL distilled water, 15 mL lemon juice, 2.25 g citric acid, and 5 g NaCl	58.6	Rastegar *et al*., 2017
	268	–	Roasting of pistachio at 150°C for 60 min by incorporation of 30 mL distilled water, 15 mL lemon juice, 2.25 g citric acid, and 5 g NaCl	47	
Unknown	–	–	Soaking of pistachio in mannan solution (25 mg/mL)	63.4	Abdolshahi *et al*., 2018
	–	–	Soaking of pistachio in mannan solution (50 mg/mL)	84.4	
Unknown	~2500	~1750	Inoculation of pistachio by *Bacillus subtilis* (UTB1) for 72 h at 37°C	30	Afsharmanesh *et al*., 2018
	~2500	~1750	Inoculation of pistachio by *Bacillus subtilis* (UTB1) and mutant (B1Δ*bacC*5) for 72 h at 37°C	30	
	~2500	~1580	Inoculation of pistachio by *Bacillus subtilis* (UTB1) and mutant (B1Δ*bacC*12) for 72 h at 37°C	36.8	
	~2500	~1000	Inoculation of pistachio by *Bacillus subtilis* (UTB1) and mutant mutant (B1Δ*bacC*18) for 72 h at 37°C	60	
Akbari	10	–	Inoculation of pistachio by *Saccharomyces cerevisiae* yeast for 12 h at 25°C	40	Rahaie *et al*., 2010a
	20	–		70	
Akbari	10	–	Inoculation of pistachio by *Saccharomyces cerevisiae* yeast for 12 h at 25°C along with acidic treatment (2M HCL)	60	
	20	–		73	
Akbari	10	–	Inoculation of pistachio by *Saccharomyces cerevisiae* yeast for 12 h at 25°C along with heat treatment (autoclave at 121°C for 20 min)	55	
	20	–		75	
Akbari	–	–	Addition of Salicylic acid solution (1 mmol/l) to medium culture	~30	Panahirad *et al*., 2014
	–	–	Addition of Salicylic acid solution (3 mmol/l) to medium culture	~42	
	–	–	Addition of Salicylic acid solution (5 mmol/l) to medium culture	~50	
	–	–	Addition of Salicylic acid solution (7 mmol/l) to medium culture	~87	
	–	–	Addition of Salicylic acid solution (9 mmol/l) to medium culture	~100	
Akbari	–	–	Addition of Salicylic acid solution (11 mmol/l) to medium culture	~100	Panahirad *et al*., 2014
Unknown	22.02**	0.48	Roasting of pistachio with salt	~98	Ostadrahimi *et al*., 2014

*Maximum tolerated level of AFB_1 following the Iranian National Standard (No. 5925, 1380), 5 µg/kg (ISIRI, 2002).
**In this research, the noted value is for TAFT
AFBB1, aflatoxin B_1.

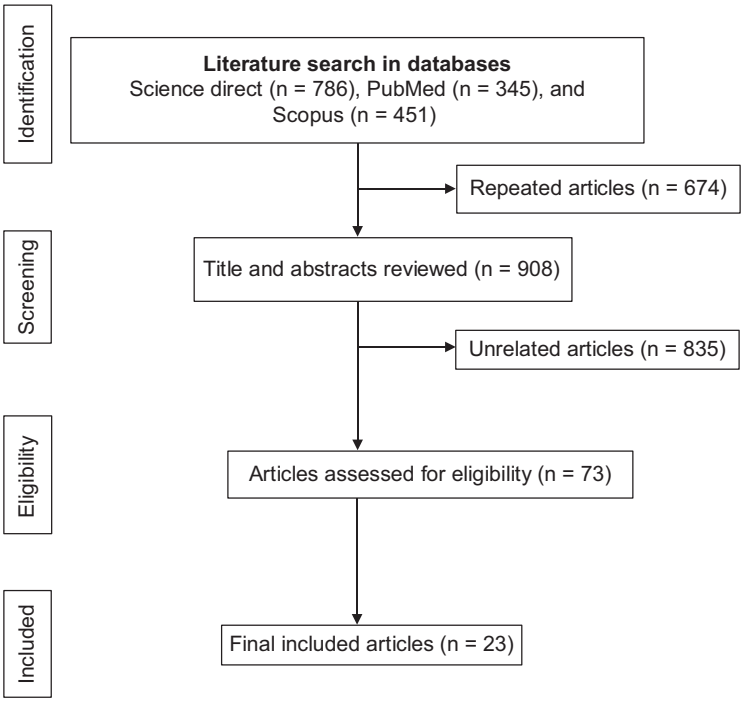

Figure 1. Flow chart of exclusion and inclusion studies based on the PRISMA guidelines.

Prevalence of AFB_1 in the Iranian pistachio

According to Table 1, the evaluation and measurement of AFB_1 content in the Iranian pistachio has been described in only 11 studies. Our work results indicate that the Iranian pistachio cultivars evaluated for AFT content include O'hadi, Akbari, Kermani, Ahmad Aghaei, Kalle-Ghuchi, and Badami. Generally, the AFB_1 concentrations of various Persian pistachio cultivars varied from <0.066 to 1,485 µg/kg (Table 1). The results demonstrated that, with time, the AFT content has decreased, probably because of increasing awareness among farmers concerning improving the sanitary conditions in the orchards, vehicles, and pistachio terminals. As per the results, among the published articles on the incidence of AFT in the Persian pistachio, only two studies reported AFB_1 content higher than 285.5 µg/kg, while in the other articles, the AFB_1 levels varied markedly from 0.066 to 10 µg/kg ($0.066 \leq AFB_1$ (µg/kg) ≤ 10). The AFT levels in ~28% of the studies were lower than 5 µg/kg ($AFB_1 \leq 5$ µg/kg), while ~35% of the studies reported concentrations in the range of $5 \leq AFB_1$ (µg/kg) ≤ 10 and ~37% of the studies mentioned levels > 10 µg/kg ($AFB_1 \geq 10$ µg/kg). As can be seen, the AFB_1 content in a significant number of studies (~72%) was higher than the acceptable limit range set by the national standard of Iran (i.e., < 5 µg/kg).

Georgiadou *et al.* (2012) reported that a critical point for AFT contamination of the pistachio nuts is the maturity step, ranging from 11 to 1,361 µg/kg among orchards. In the harvesting step, the AFT content was >1,420 µg/kg, and the authors concluded that the higher AFT levels (>1000 µg/kg) in orchards correlated with the highest insect infestations during this production step. At the postharvest level, AFT contamination in three out of four orchards varied from 650 to 1,100 µg/kg. During the storage period, it ranged between 40 and 1,200 µg/kg.

Considering that some environmental factors are needed to support fungal growth, such as temperature and the relative humidity of the environment and storage, proper drying and suitable storage of grains are the most important preventive actions to prevent fungal growth. In this context, fungal contamination and the subsequent AFT production in pistachio nuts may occur in orchards and farms, at harvesting time, during transportation, during the process stage, and storage, and as well as when distributing the product (Cheraghali *et al.*, 2007).

In this study, 23 articles were found related to AFT's prevalence in the Iranian pistachio, which indicated that the most frequent AFT found in pistachio is AFB_1. Several studies have confirmed the presence of AFB_1 in the Iranian pistachio (Afsharmanesh *et al.*, 2018; Panahirad *et al.*, 2014; Rahaie *et al.*, 2010a; Yazdanpanah *et al.*, 2005). Dini *et al.* (2013) studied the AFT contamination levels in the Iranian pistachio and concluded that

the mean AFB_1 levels in this product in 2009, 2010, and 2011 were ~320 246, and ~370 µg/kg, respectively. They also observed that the measured AFT was higher than those recommended by the Iranian National Standard in all the years mentioned above. In another study, Cheraghali et al. (2007) claimed that AFB_1 content in ~63.3% of the pistachio samples was lower than the limit of detection (LOD) of the analytical method used in the study (5 µg/kg).

Analytical methods for the determination of AFT

Today, the successful development of fast, robust, cheap, and simple analytical methods with high selectivity and sensitivity for measuring and identifying different mycotoxins in different foods products is a reality (Alshannaq and Yu, 2017; Zhang *et al*., 2018). New methods rely on gas and high-performance liquid chromatography (HPLC) systems combined with tandem mass spectrometry (MS/MS), thereby providing an unequivocal identification and precise quantification of multiple mycotoxins in complex food matrices (Turner *et al*., 2015). Also, HPLC coupled with fluorescence detection (FLD) was developed as one of the nonMS methods earlier by the European Standardization Committee (CEN) and the Association of Official Analytical Chemists (AOAC) International for the determination and identification of different mycotoxins (Kwaśniewska *et al.*, 2015; Pascale, 2009; Pereira *et al*., 2014). The HPLC-FLD sensitivity is comparable to the results determined by the HPLC-MS/MS technique, but the HPLC-FLD is usually more suitable for single mycotoxin detection or the determination of mycotoxins group with similar chemical structures (Khaneghah *et al*., 2019). In all the literature studied (Figure 2a), the analytical methods based on HPLC were the most used for the determination of AFTs in the pistachio nuts, followed by thin-layer chromatography (TLC) and enzyme-linked immunosorbent assay (ELISA).

The ELISA method is based on the chemical interaction between a given antigen (in this case, a mycotoxin conjugated to the larger, immunogenic molecule) and its specific antibody. According to this interaction arrangement, ELISA protocols can be designed as direct, indirect, sandwich ELISA, competitive, or indirect competitive. In the

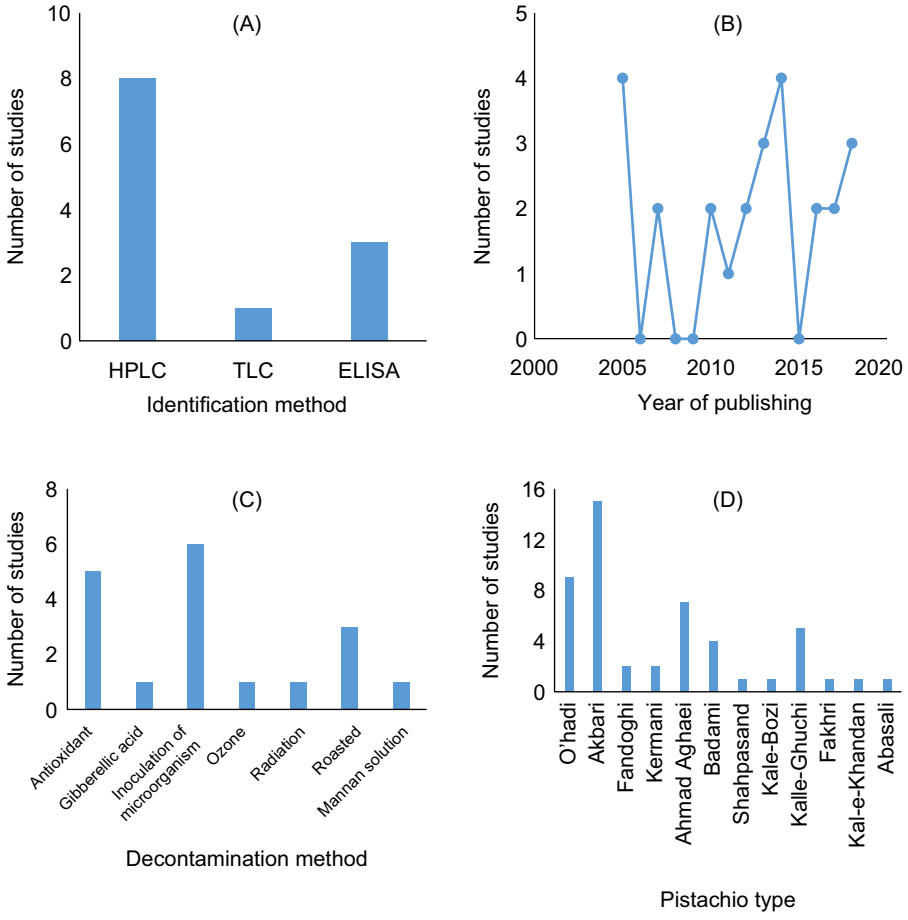

Figure 2. The number of articles and studies on identification methods (A), year of publishing (D), the decontamination method (C), and the pistachio type (D).

direct ELISA, the extract of samples reacts directly with the enzyme, thus providing rapid and beneficial results for mycotoxins' qualitative evaluation. Competitive ELISA shows the competitive reaction between sample (antibody or antigen) and target mycotoxin in the enzyme-labeled targets versus immobilized antibody or antigen. The indirect technique is based on indirectly determined target antigen using the secondary antibody, which is usually labeled as an enzyme. The indirect competitive technique's mechanism is the interaction of the primary antibody with the immobilized antigen, and in this method, a secondary antibody conjugated with an enzyme is required. In the sandwich technique, the antigen interacts with two different antibodies (Suzuki *et al*., 2007), which allows high specificity, rapid speed screening, simple operation without using any readers or instrument, and eco-friendly analysis due to radioactive labels and low volumes of solvents (Batrinou *et al*., 2020; Krska and Molinelli, 2009; Sakamoto *et al*., 2018).

One of the most critical limitations of the regular monitoring of mycotoxins in foods using ELISA is the high price of commercially available kits, especially in developing countries (Rossi *et al*., 2012). The further investments in immunoreagent manufacturing could increase the access to the kits as ELISA is the most common immunoassay applied for fast mycotoxin determination. Other fast immunoassay-based methods, including radioimmunoassay, immunoaffinity columns, and sequential injection immunoassay, have been applied to identify and determine mycotoxins in food products. Moreover, rapid, on-site test bands have been developed to measure toxic contaminants in foodstuffs, including a flow-through, lateral-flow immunoassay (LFIA), and dipstick. The lateral flow-based essay is a big, sensitive, selective, and single-step test for determining different mycotoxins in about 10 min. This technique is based on three factors: a porous membrane, an absorbent pad, and a conjugate pad. These three factors ensure a lateral flow, increase the flowing liquid volume, and guarantee contact between the liquid sample and the membrane (Maragos and Busman, 2010).

Methods for the decontamination of AFTs in the Iranian pistachio

The experimental techniques applied for AFT decontamination of the pistachio are presented in Table 2. It is noteworthy that the majority of the studies were conducted using Iranian pistachio cultivars such as O'hadi, Ahmad Aghaei, Akbari, Shahpasand, Abasali, Kale-Bozi, Kalle-Ghuchi, FAS-13-73, Fakhri, and Kal-e-Khandan (Table 2, Figure 2b and d). In accordance to the results reported in the evaluated studies, the lowest (2.46%) and the highest (100%) percentages of AFB_1 removal were related to treatments using "pistachio Testa (pistachio kernel coat)" and "addition of 9 mmol/l Salicylic acid solution to the medium culture," respectively. Overall, the applied methods for the decontamination of AFTs in the Iranian pistachio included physical (radiation and roasting) and chemical methods (gibberellic acid, treatment with ozone, lemon extract, citric acid, salt solution, and salicylic acid solution). While biological treatments, such as *Saccharomyces cerevisiae*, *Trichoderma* extracellular extract, inoculation by *Bacillus* species, and recombinant mutants of *Bacillus subtilis*, attracted notable attention, combined methods and the use of extract and essential oils from medical plants, such as phenolic compounds from pistachio green hull, the extract of the Shirazi thyme, and Testa or the pistachio kernel coat, were also useful (Figure 2c).

Abdolshahi *et al.* (2018) studied the AFT-binding efficiency of *S. cerevisiae* mannoprotein (mannan) in contaminated pistachio nuts, observing the highest binding percentage (84.4%) for AFB_1 at a higher concentration of mannan (50 mg/mL). In another research, the simultaneous application of roasting-chemical treatment (lemon juice and citric acid) for the reduction of AFB_1 in pistachio was evaluated by Rastegar *et al.* (2017). The authors found that roasting of pistachio nuts (50 g) in water (30 mL), lemon juice (30 mL), and citric acid (6 g) at 120°C for 60 min resulted in a significant reduction (93.1%) of AFB_1. Also, the roasting of pistachio nuts (50 g) with water (30 mL), lemon juice (15 mL), and citric acid (2.25 g) at 120 C for 60 min decreased the level of AFB_1 to 49.2% of the original content. Generally, it was reported that the simultaneous use of heat and citric acid/lemon juice had a synergistic effect on AFB_1 reduction. In summary, physical, chemical, and biological methods, as proposed in several studies, could significantly reduce the pistachio nuts' AFT contents. However, methods based on the inoculation of different microorganisms that are generally recognized as safe (GRAS), such as *B. subtilis* and *S. cerevisiae*, have a higher potential for practical application pistachio industry. Siahmoshteh *et al.* (2017) demonstrated that both strains could decrease the mycelial growth *in vitro*, reducing the production of AFB_1, AFB_2, AFG_1, and AFG_2 during the first 3 days after inoculation.

Conclusions

The results of this study indicate that the occurrence levels of AFB_1 in the Persian pistachio cultivars are a public health concern as 72% of the studies reported concentrations higher than the acceptable level per the Iranian National Standard for this product. The high incidence of AFTs in the Iranian pistachio is probably due to a lack of knowledge among the farmers, pistachio terminals, and the pistachio processing stages on the preventive

measures with regard to toxigenic fungi contamination of foodstuffs. Therefore, further preventive actions and also ongoing monitoring can counter the potential risk of AFT in pistachios. Although several experimental techniques have been proposed for the reduction of the AFT content in pistachio nuts, the inoculation of GRAS microorganisms shows the most significant potential for future applications in the pistachio industry.

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