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Review Article

The Effect of Lactobacillus Strains on Aflatoxin M1 Residues in Dairy Products: A Systematic Review

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ABSTRACT

Background: Aflatoxin M1 or milk toxin has been detected in many parts of the world both in raw milk and dairy products. It has been shown that the lactic acid bacteria especially *Lactobacillus spp.* reduce aflatoxin M1 levels in dairy products.

Methods: We performed a systematic review to evaluate the effects of Lactobacillus strains on aflatoxin M1 residues. So a systematic literature search by using certain keywords was carried out in three bibliographic databases on aflatoxin M1 binding ability of Lactobacillus species in milk and dairy products. After the initial screening of the titles and abstracts, the related articles to our work were retrieved and the full text of the studies, which probably included the required data were obtained. The eligible articles were selected based on the inclusion criteria mentioned in the methodology.

Results: In general, *Lactobacillus spp.* was shown to have a potential application to decrease the aflatoxin M1 levels in milk and dairy products from less than 10% to up to 99%. Also, this systematic review revealed that the reducing effect of *Lactobacillus spp.* on aflatoxin M1 residues was dependent on several factors including fermentation, incubation and storage time, bacterial population, type and viability of bacteria, and concentration of aflatoxin.

Conclusion: Application of Lactobacillus strains in production of the dairy products from contaminated milk can be a very effective way to reduce aflatoxin M1 level in these products.

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Introduction

Aflatoxins are one of the most potent and dangerous groups of mycotoxins produced by various fungi, especially *Aspergillus flavus* and *Aspergillus parasiticus*. These fungal species contaminate a variety of foods and agriculture products such as wheat, barley, rice, oats and cereal grains particularly maize (1). Aflatoxins have been classified as group 1 carcinogens by International Agency for Research on Cancer (IARC) (2) and among the naturally occurring aflatoxins, aflatoxin B1 (AFB1) is the most carcinogenic one (3). When cows are fed with contaminated feed, aflatoxin B1 is converted to aflatoxin M1 (AFM1) during the hydroxylation in the liver, which is subsequently secreted in the milk of lactating cows (4). AFM1 is

less poisonous than aflatoxin B1, however, because of its carcinogenic and hepatotoxic effects, AFM1 is also classified as group 1 carcinogenic compounds for humans by IARC (3). To effectively minimize and control aflatoxin M1 levels, preventive practices are the only safe ways to avoid AFM1 contamination undertaken throughout the production of an animal feed or forage.

Milk and dairy products are known as an important source of nutrients such as protein and calcium that help blood pressure reduction or prevent colon cancer. Since consumption of these products is high, the contamination of this valuable foodstuff and its products is considered as a serious risk to the general health of the community especially in under-developed countries (5, 6). AFM1 is resistant against high temperatures and is not significantly reduced during the thermal processes such as pasteurization, sterilization, and other food processing procedures (7, 8). Thus, an effective and practical method is essential to remove or minimize AFM1 residue in contaminated products (9, 10). Different papers have studied biological methods such as aflatoxin binding capacity to lactic acid bacteria (LAB) (11, 12).

Potential of Lactobacillus strains in aflatoxin risk mitigation have been studied by several researchers. Several studies have shown that a reduction in AFM1 level can happen due to inoculating of milk by lactic acid bacteria strains. These studies also showed that reduction of aflatoxin level can occur during a physical binding between the cell wall of bacteria and aflatoxin molecule, and it seems that LAB strains have no considerable damaging effect on aflatoxin molecules too. Therefore, the decline is observed only in the level of free aflatoxin molecules. When a comparison between the toxic effects of free aflatoxin molecules and aflatoxin molecules binding to the bacteria cell wall was conducted, it was demonstrated that concurrent administration of some species of LAB strains or probiotics with aflatoxin M1 or B1 could strongly reduce the bioavailability and adverse effects of aflatoxin molecules (11, 13-17). Several researchers have also investigated the influence of different parameters on AFM1 binding ability of Lactobacillus strains such as initial amount of aflatoxin, time and temperature of incubation, pH and type of used culture (1, 18, 19). However, no systematic review has ever been conducted. So, the aim of this study was monitoring the impact of Lactobacillus strains on AFM1 residues in dairy products.

Materials and Methods

Search Strategy

A systematic literature search was carried out in three bibliographic databases (PubMed, Scopus, Science Direct and Google Scholar) on AFM1 binding ability of *Lactobacillus spp.* in dairy products. The following keywords were used for the search in title, abstract and keywords; (lactic or LAB or probiotic or lactobacillus) and (aflatoxin or mycotoxin) and (dairy or cheese or milk or yogurt or butter or product or food) until April 20, 2018. After screening of the titles and abstracts, the irrelevant articles were excluded and only the related articles to our work were retrieved. To find the papers that might have been missed out during the search, the reference sections of the related articles were checked. Only AFM1 binding ability to *Lactobacillus spp.* was considered and the articles related to the other lactic acid bacteria or the other microorganisms were excluded. Articles containing none-English-language text, the review articles, and book chapters were also excluded. Articles containing antifungal activities of Lactobacillus strains and the studies which were related to the effect of a mixture of Lactobacillus strains on the reduction rate of AFM1 were excluded too. The full text of the studies, which probably included the required data were obtained. Thereafter, the eligible articles were selected.

Data Extraction and Inclusion Criteria

The information including authors, year of publication, the initial concentration of AFM1, viability status of the bacterial cells, type of bacteria (just *Lactobacillus spp.*), the percentage of AFM1 reduction, kind of samples were extracted. Results related to the AFM1 biding potential of Lactobacillus strain individually, in milk or the other dairy products such as yogurt and kefir were also considered.

Literature Search and Data Extraction

Following by research in three global databases based on the mentioned keywords, 603 articles were found. After exclusion of the duplicate articles, 422 publications remained. The remained papers were assessed based on titles and abstracts. After screening by titles, 354 articles were excluded and 68 papers were included. By abstract screening, 31 publications with relevance abstracts were retrieved for preparing full texts and were further assessed for eligibility. Subsequently, the studies not related to our study were excluded. Finally, 12 publications were eligible to be enrolled in our study based on the inclusion criteria mentioned in the methodology. The data collected from the selected studies belonged to *Lactobacillus spp.* including *L. acidophilus*, *L. rhamnosus*, *L. plantarum*, *L. lactic*, *L. helveticus*, *L. bulgaricus*, and *L. casei*. All studies determined AFM1 content via HPLC equipped

with a fluorescence detection system at excitation and emission wave lengths between 365 and 435 nm, respectively, but also some of them used the enzyme-linked immunosorbent assay (ELISA) for the analysis. Information of the selected articles was summarized in Table 1. A flow diagram with the details of the study selection and search strategy was shown in Figure 1.

Results and Discussion

Review of the studies on individual Lactobacillus species showed that *Lactobacillus spp.* can be effective in reducing AFM1 levels in dairy products over 99%, but this effect is probably the sum of multiple individual factors (20). The percentage of AFM1 reduction in fermented and non-fermented products was significantly different. Factors such as viability, type of bacteria, initial concentration of aflatoxin, and fermentation conditions such as contact time were considered as effective factors. Mechanism of AFM1 reduction by lactic acid bacteria is not still clarified. However, it has been suggested that aflatoxin molecules are removed through a non-covalent binding to bacterial cell-wall components mainly to polysaccharides, peptidoglycans and also teichoic acids instead of degradation by bacterial metabolism. It seems that the stability and strength of binding of bacteria to aflatoxins are dependent on type of the bacteria, cell wall component, and environmental conditions. Lactobacillus strains seem to be able to create a more stable connection than other lactic acid bacteria (21-24).

Effect of Fermentation

Several studies investigated the effect of different types of *Lactobacillus spp.* on aflatoxin levels in fermented products such as yogurt, kefir or milk, and determined the reduction rate of AFM1 during the fermentation and storage of such products alone and in presence of a certain Lactobacillus strains. Elsanhoty *et al.* investigated the reduction rate of AFM1 level during the processing and storage of yogurt (alone), yogurt with *L. plantarum* and yogurt

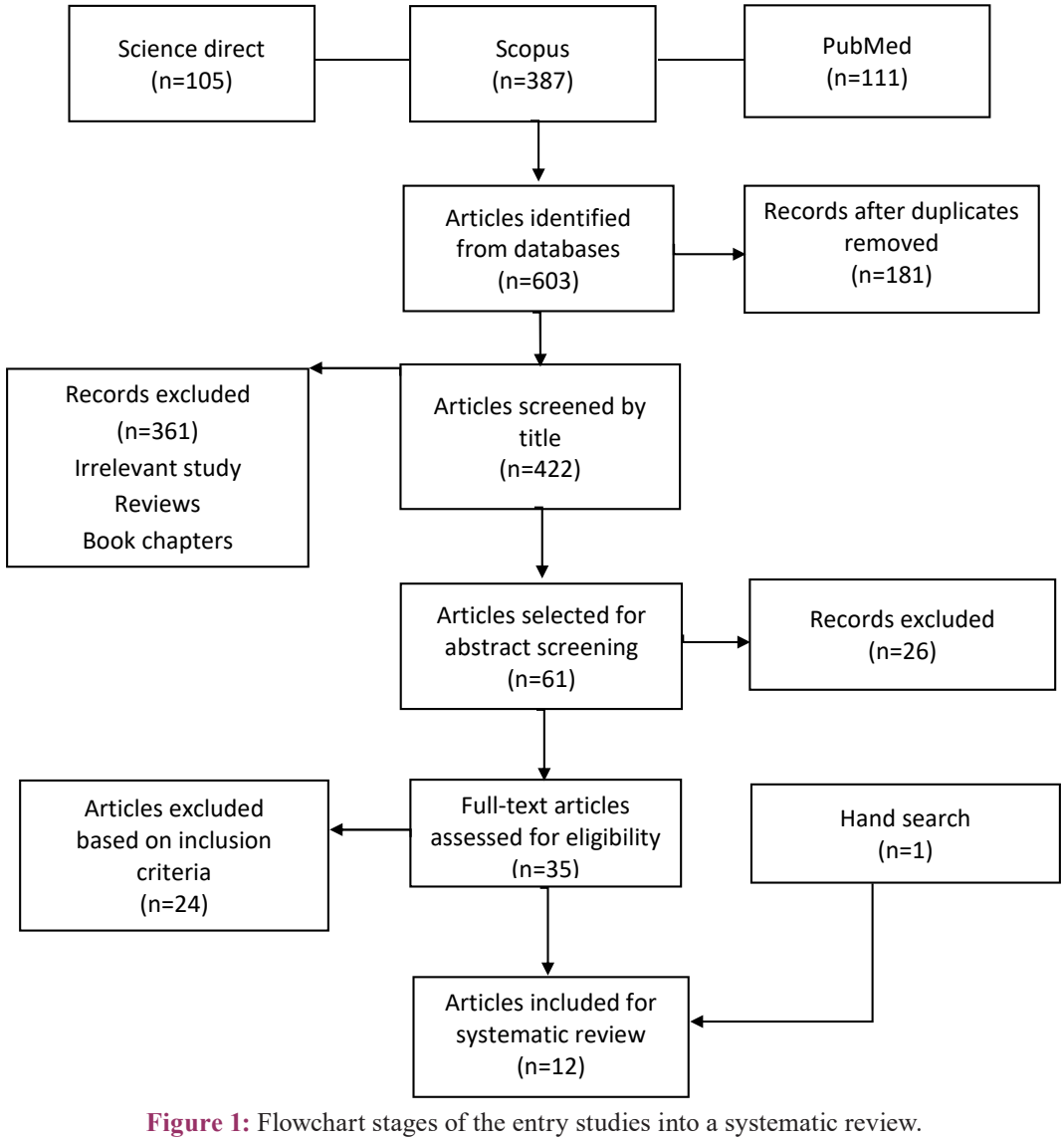

Figure 1: Flowchart stages of the entry studies into a systematic review.

Table 1: Results of reviewed studies.

Microorganism	Product	Viability	Initial concentration of AFM1 (µg/L)	Population (Log)	Contact time	Percentage of AFM1 removal (Mean)	Reference
L. casei	Fermented milk	Viable	0.05	7	21d	58	(18)
L. acidophilus	Doogh (Iranian fermented milk)	Heat treated	0.5	7	1-28 d	64-78.4	(20)
		Viable		7	1-28 d	51.2-95.2	
				9		61-99	
L. casei		Viable		7	1-28 d	38-50.2	
L. rhamnosus		Viable		7	1-28 d	39.8-56.4	
L. acidophilus	Yogurt	Viable	0.1	8	1-21d	85.58-91.76	(17)
			0.5			91.89-92.7	
			0.75			95.02-97.12	
	Milk		0.1		1-21d	91.18-95.44	
			0.5			92.96-94.44	
			0.75			97.8-99.8	
L. acidophilus	Yogurt	Viable	50	6	1-7d	27.8-72.8	(16)
L. plantarum					1-7d	31.5-87.8	
L. casei	Milk	Viable	0.5	-	48h	82.12	(25)
	Kefir			-		69.19	
L. bulgaricus	Fermented milk	Viable	0.05	6	2-6h	46.1-58.5	(19)
L. rhamnosus GG	Skim milk	Viable	0.1	8	16h	18.8	(31)
	Full cream milk					26	
		Heat treated				26.6	
						36.6	
L. rhamnosus LC-705	Skim milk	Viable				69.6	
	Full cream milk					63.6	
		Heat treated				27.4	
						30.1	
L. acidophilus NCC 12	Reconstituted milk	Viable	5	8	4h	15.44	(28)
			10			14.84	
			20			14.37	
		Heat treated	5			19.02	
			10			16.6	
			20			18.96	
L. acidophilus NCC 36		Viable	5			22.7	
			10			21.76	
			20			22.47	
		Heat treated	5			23.73	
			10			24.13	
			20			25.07	
L. acidophilus NCC 68		Viable	5			9.55	

			10			7.85	
			20			10.51	
		Heat treated	5			15.36	
			10			12.85	
			20			15.92	
L. rhamnosus		Viable	5			21.74	
			10			22.14	
			20			20.41	
		Heat treated	5			25.13	
			10			22.86	
			20			26.27	
L. rhamnosus	Milk	Heat treated	0.5	10	15h	24.46	(27)
L. bulgaricus						33.54	
L. helveticus	Milk	Heat treated	0.05	7-10	1h	36-100	(26)
			0.1			26-85	
L. lactic			0.05			26-76	
			0.1			19-73	
L. plantarum			0.05			18-80	
			0.1			13-77	
L. rhamnosus	Reconstituted milk	Viable	0.05	8	0-24h	25.1-85.8	(12)
			0.1			26.1-90.7	
			0.2			25.3-95.1	
L. plantarum			0.05		0-24h	15.3-72.3	
			0.1			15.8-72.9	
L. bulgaricus	Milk	Viable	10	-	4h	27.56	(23)

with L. *acidophilus*; while the degradation levels of AFM1 in these products were reported to be 61.4%, 89.9%, and 84.8%, respectively (20). Adibpour *et al.* have also found that the reduction rate of AFM1 levels in yoghurt with *L. acidophillus* (around 99%) was higher than yogurt when compared with yogurt culture alone (21). Similar results were observed by Tajalli *et al.* and also Sarlak *et al.* who reported AFM1 reduction levels to be around 37-39% in doogh (a traditional Iranian fermented milk with yogurt culture alone) and around 95% in presence of *L. acidophilus* (25, 26).

It seems that yogurt culture alone had a less impact on AFM1 level in comparison to Lactobacillus strains or even did not have a significant reduction impact on AFM1 level. So as Blanco *et al.* demonstrated no reduction in AFM1 concentration in the yoghurt when compared with yoghurt culture alone during the storage period (27). Sarimehmetoglu *et al.* have reported yogurt culture alone to decrease AFM1 level less than pure cultures of *Streptococcus thermophilus* and *L. bulgaricus*, while illustrating a reduction level of 14.82%, 27.56%, and 39.16% for AFM1, respectively (28). El khoury *et al.* noticed a similar result with the same differences. They found combined culture of *L. bulgaricus* and *S. thermophilus* was more effective than pure culture of *S. thermophilus* (23). Barukcic *et al.* noted identical findings on yogurt and kefir cultures and reported all treatments with probiotic cultures to be more effective. In their study, the kefir starter alone was the least efficient in all tested cultures and *L. casei* was recognized as the most efficient strain, achieving a reduction level of approximately 58% (29). However, Sani *et al.* reported a different result for kefir culture. They showed that the reduction rate of AFM1 in kefir and kefir culture alone (85%) was more prominent than the fermented milk by combination of kefir culture and *L. casei* (81.76%) and also fermented milk by *L. casei* (69.19%) (30).

Among all studies related to fermented products, the highest reduction rate was reported by Adibpour *et al.* employing *L. acidophillus* with a population of 10^8 cfu/mL that could reduce AFM1 level in fermented milk more than 99% (21). Ismail *et al.* have also reported a high reduction rate of about 81% in milk by adding *L. casei* (31). However, as shown in Table 1, the other studies that were performed during a short

incubation or storage time, with a population less than 10^8 cfu/mL; and without fermentation, indicated a low reduction rate in AFM1 level (28, 31-33). According to the results of the previous studies, Lactobacillus strains can bind the aflatoxin molecules probably through a physical adhesion. The effect of fermentation on AFM1 in dairy products can be mainly attributed to the changes that occur during the fermentation, so that in this situation, milk proteins such as casein are denatured and more hydrophobic sites are exposed that can bind more to aflatoxin molecules (20, 22, 25, 34).

Elsanhoty *et al.* found that decreasing of pH during 7 days of storage can lead to more reduction in AFM1 level in milk, so development of organic acids and a reduction in pH can cause alteration in the structure of caseins and protein components. These changes can lead to the formation of a network like yogurt coagulum that holds the aflatoxin inside the precipitate (20). Some authors have demonstrated the effect of dairy components on AFM1 removal in comparison to the effect of Lactobacillus strains on AFM1 level in dairy products and in phosphate buffered saline (PBS) medium. El Khoury *et al.* found that LAB cultures removed higher levels of AFM1 in skimmed milk when compared to PBS; so after 2 hours of incubation, they reported AFM1 degradation in yogurt made by pure culture of *L. bulgaricus* and PBS about 46.1% and 38.7%, respectively (23). Sarimehmetoglu *et al.* found a similar result and reported percentages of AFM1 removal around 18.7% and 27.7% for *L. bulgaricus* in PBS and yogurt, respectively (28).

However, Abbes *et al.* showed no significant difference between reduction rate of AFM1 by the tested bacteria in PBS and reconstitute milk depending on the contamination level and incubation period. They reported a range of AFM1 removal about 16.1-78.6% and 15.3-76.9% for *L. plantarum* and about 26.2-86.6% and 25.1-95.1% for *L. rhamnosus* in PBS medium and reconstitute milk, respectively (11). Kabak and Var also observed no significant difference between AFM1 binding of all heat-killed and viable bacteria in PBS and skim milk except for one strain of *L. acidophilus* with the binding ability of 19.29% and 12.85% in PBS and skim milk, respectively (33). Regardless of differences in AFM1 binding ability of different *Lactobacillus spp.* and the behavior of starter cultures used, the reason for these contradictions probably may be due to factors such as fermentation condition or population of bacteria; so the removal percentage of AFM1 has been dependent on factors varied from less than 10% to 100%. All data and results of these 12 papers were shown in Table 1.

Effect of Viability

Several researchers have investigated the effect of heat inactivation of Lactobacillus strains on AFM1 binding capacity. In all reviewed papers, seven studies were related to the viable form of bacteria, two investigations were conducted on heat-treated bacteria and three were evaluating both. Some surveys have monitored the binding ability of bacteria in initial hours of exposure and reported heat treatment of the bacterial cells to improve the aflatoxin binding possibly via protein denaturation and increase in hydrophobic nature of surface or formation of Maillard reaction products (25, 33, 35). Probably, such changes led to binding of aflatoxin molecules to the plasmatic membrane and bacterial cell wall components which were inaccessible when the cell wall was intact (36-38). However, the studies on heat-treated bacteria that were performed in non-fermeted products indicated degradation percentages of less than 50% in a population of bacteria with less than 10^8 cfu/mL (31, 32, 37). Some researchers compared the binding potential of both forms of viable and heat-killed bacteria and reported different results. Kabak and Var assessed six dairy strains of Lactobacillus and Bifidobacterium and found no significant differences between heat-treated and viable bacteria for low concentration levels of AFM1 (5 and 10 µg/mL). In general within 4 hours, the reduction rate of the heat-treated bacteria was a bit more than the viable cells; but the difference was significant for the strain of *L. acidophilus* at a level of 20 µg/mL (33, 39).

Assaf *et al.* investigated the effects of some treatments such as washing, pipetting, and heating on AFM1 bound to the bacteria (*L. rhamnosus* GG) in AFM1 solution after 18 hours incubation. They observed that pipetting and centrifugation of the suspension of bacteria and AFM1 solution (till complete homogenization) after a heat-treatment and before incubation could improve the binding ability of AFM1 and also reported AFM1 binding percentages of 56.43% and 55.62% for viable bacteria that enhanced to 58.86% and 63.08% for heat-treated bacteria without and with pipetting, respectively (35). Sarlak *et al.* noticed at the first day, the binding potential of heat-treated *L. acidophilus* in doogh to be more than the viable form of bacteria with a reduction rate of 64% and 51.2%, respectively. After 21 days, the percentage of AFM1 removal by viable form of bacteria was significantly higher than killed-bacteria with a reduction rate of 95% and around 78%, respectively (25).

According to the results of the comparative studies on AFM1 binding potential for viable and heat-treated bacterial cells, it seems that in initial

hours of the storage, AFM1 binding potential of heat-killed bacteria to be more than the viable bacteria; but, at the end of the storage period, viable bacteria were more effective in reduction of AFM1 level (25, 36, 37). High percentages of AFM1 removal by heat-treated bacteria have been reported by many researchers including Ismail *et al.* who applied killed bacteria in a high population rate (more than 10^8 cfu/mL) (31) and Sarlak *et al.* who evaluated the effect of bacteria on contaminated fermented products and in a long storage time (25). A correlation was shown between two variables of population of bacteria and the contact time with binding potential of viable bacteria (Table 1) (11, 15, 16, 19).

Effect of Bacterial Population

All studies that assessed AFM1 binding potential of heat-treated bacteria revealed that in a bacterial concentration of less than 10^8 cfu/mL, the percentages of AFM1 removal were less than 50% (31, 32, 37). Ismail *et al.* found that the population of bacteria was very important for the potential of the heat treated-bacteria. They observed that the reduction rate was around 30% for concentration of 10^8 cfu/mL depending on the bacterial type that could be enhanced to 80% or even 100% (for *L. helveticus*) for the concentration of 10^{10} cfu/mL (31). However, Bovo *et al.* did no't find a reduction rate of more than 35% for *L. bulgaricus* and *L. rhamnosus* even for the bacterial concentration of 10^{10} cfu/mL (32). Kabak and Var assessed the effect of bacterial population on AFM1 binding level in PBS and found that the tested bacteria for the population of 10^8 cfu/mL could decrease AFM1 level from 10.22 to 26.65% depending on contamination level and incubation period; while for the population of 10^7cfu/mL dropped to 0-5.02% (33). Sarlak *et al.* reported a similar result and found the ability of viable *L. acidophilus* in AFM1 binding ranging from 51.2% to 95.2% (from day1 to day 28) for the population of 10^7cfu/mL that improved to 61-99% for the population of 10^9cfu/mL (19). The reason for these findings was mentioned as increase in the number of bacterial cells, and physical adhesion between the bacteria and AFM1 becoming stronger.

Effect of Incubation Time and Storage Period

Several studies have investigated the effect of different contact times on AFM1 binding ability to the bacteria. In some studies, the contact time varied from initial hours of exposure for non-fermented products to 4 weeks for fermented products. The results of these studies showed that the time was an effective factor for the binding ability of the bacteria. Among all studies, eight papers investigated the binding potential of the viable and heat-treated Lactobacillus species during a short period of time (a variety of time between 0 to 24 hours) (11, 18, 28, 31-33, 37). They reported a low percentage of AFM1 removal in comparison to those during a longer period of time. Abbes *et al.* compared the effect of different incubation time (0h, 6h, and 24h). They revealed a direct correlation between the incubation time and elimination of AFM1 revealing an increase about 60-70% from 0 h to 24 h depending on the bacterial type and initial AFM1 level. El Khoury *et al.* have reached similar results reporting the lost percentages from 46.1% during 2 h incubation period that increased to 58.5% during 6 h incubation period (11, 18). Some assessments were conducted for a storage time more than 24 hours (a variety of time between 1 day to 21 days) (15-17, 25, 30).

In general, these studies reported high removal percentages about 50% and over 99%. Some papers evaluated the effect of different storage time and revealed similar results reporting.023 that binding percentages of different strains of *Lactobacillus spp.* increased during the storage period and the most extensive reduction was observed at the end of storage period. Elsanhoty *et al.* found after 7 days storage of yogurt, a reduction in AFM1 about 45% and 56% for *L. acidophilus* and *L. plantarum*, respectively (15). However, Adibpour *et al.* investigated the AFM1 binding ability of *L. acidophilus* strain in the presence and absence of yogurt starter culture and yogurt starter culture alone during the storage period of 21 days in the refrigerator. They observed a degradation percentage of more than 90% for AFM1 level among all groups at the first day of storage; while no significant increase was observed in degradation level during the storage time. The most changes in AFM1 removal during the storage time belonged to the yogurt culture alone revealing at the end of the first week to reach to the highest level (16). Sarlak *et al.* compared the binding potential of heat-killed bacteria and viable bacteria during the storage time. The increase in storage time was more effective on the binding potential of the viable bacteria when compared to the heat-killed bacteria (Table 1). They found that the reduction rate significantly increased on days 14 and 28 in comparison to the first day of both groups. But the amount of reduction level of AFM1 by viable bacteria was more than the heat-killed bacteria (with an increase of about 40% and 14%, respectively) (20). The differences for AFM1 binding ability between the heat-treated and viable bacteria is probably due to the reproducing activity of the bacteria during the storage time and consequently their effects on the nature of products during the storage time and also

during the fermentation period (25).

Effect of Bacterial Type

Because of the different designs in various researches, performing an exact comparison on binding ability of the strains in different studies was not possible. Potential of bacterial strains in AFM1 reduction probably depends on some factors such as AFM1 binding capacity of bacteria or stability of the AFM1/bacteria complex. Several researchers have assessed the capacity of different types of bacteria regarding the binding of AFM1. According to the previous investigations on all bacteria, Lactobacillus strains seem to have more AFM1 binding potential. El khoury compared the AFM1 binding ability of *L. bulgaricus* and *S. thermophilus* separately and their combination revealing the arrangement of *L. bulgaricus* combined culture combined culture>*S. thermophilus* with binding levels of 58.5%, 46.7, and 37.5, respectively (18). Sarlak *et al.* have also compared three Lactobacillus species and reported *L. acidophilus* (with 95.2% reduction after 28 days) to be more efficient in reduction of toxin levels than *L. rhamnosus* and *L. rhamnosus* (56.4%) and *L. casei* (50.2%) (19). Elsanhoty *et al.* showed that *L. plantarum* (with a reduction of 87.8% after 7 days) was more effective than *L. acidophilus* (72.8%) in yogurt (15). In comparison of the binding ability of different strains of a specie of *Lactobacillus spp.*, Pierides *et al.* demonstrated a significant difference of 50% between binding potential of two strains of *L. rhamnosus* in viable form of bacteria regarding GG and LC705; while this difference for heat-treated form of the bacteria was not visible. They found that the type of bacteria even for closely related strains may have different biological activities (37). Kabak and Var compared different strains of *L. acidophilus* and concluded absence of any significant difference between the ability of the different strains to remove AFM1 (32).

Some scientists also have studied the stability of AFM1/bacteria complex by determining the amount of bounded AFM1 to the bacteria after repeated washes (11, 33) and illustrated that the binding was not irreversible and small amounts of AFM1 were released back into the medium (35). Kabak and Var reported an amount of 5.62-8.54% of bounded AFM1 by the bacteria to be released back into the buffered solution (33). Serrano-Niño *et al.* have also exhibited 1.46%-4.37% release percentage for five species of Lactobacillus; while the highest percentages belonged to *L. reuteri* and *L. rhamnosus*, respectively (14). Ismail et al. studied heat-treated forms of the bacteria and recognized the LAB spices could form the most stable AFM1 complex among all tested bacteria and also found *L. helveticus* (in a population of 10 log/mL) as the strain with the highest binding potential among all the strains of Lactobacillus (31). Assaf *et al.* showed that application of pipetting and heating could increase the binding of AFM1 with no improvement in the stability of the complex (35). Generally, in the same experimental conditions, the higher removal percentages belonged to *L. acidophilus* and *L. rhamnosus*, respectively.

Effect of Initial Concentration of AFM1

The dairy products used in all studies were artificially contaminated with certain amounts of AFM1. Several researchers have investigated the correlation between the initial concentration of AFM1 and the reduction rate. However, they have reported different and even contradictory findings. Kabak and Var compared AFM1 binding ability of *L. rhamnosus* and three strains of *L. acidophilus* in spiked reconstituted milk and found no significant correlation between the initial concentration and removal of AFM1 levels (33) Abbes *et al.* evaluated AFM1 binding ability of two strains of *L. rhamnosus* and *L. plantarum* in three levels of initial AFM1 and three incubation time (0h, 6h, and 24h) and reported binding ability of 85.8%, 90.7% and, 95.1% for *L. rhamnosus* respectively and 72.3%, 72.9%, and 76.9% for *L. plantarum* in AFM1 concentration of 0.05, 0.1, and 2 µg/L, respectively (11). Adibpour *et al.* reported the binding ability of Lactobacillus strains to increase by a rise in initial AFM1 concentration (16). However, Ismail *et al.* found the AFM_1 binding potential of Lactobacillus strains in lower initial concentration levels to be more than their AFM1 binding ability in higher concentration levels (31). These contradictions may be described by the differences in the experimental conditions and procedures (15).

Immunological Effect

Because of LAB can eliminate aflatoxins physically and reduces the free aflatoxins, some researchers have compared the toxicity of free aflatoxins and bounded aflatoxins. Serrano-Niño assessed the ability of some probiotic strains to reduce the bioaccessibility of AFM1 using a digestive model and demonstrated that the bioaccessibility of the toxin decreased about 22.7-32.2% for Lactobacillus strains and also 45.17% for *Bifidobacterium bifidum* (14). Abbes *et al.* during an *in vitro* study noticed that the general toxicity of AFM1 significantly decreased in the AFM1-treated mice when the mice received AFM1-bacteria complex (11). Jebali *et al.* have also reported the same results revealing adverse effects for both AFM1 and AFB1 to be decreased due to the co-treatment with *L.*

plantarum. So it seems that the effect of Lactobacillus strains on the reduction of aflatoxins toxicity may be probably due to a decrease in the bioaccessibility of the mycotoxin molecules (13).

Conclusion

The results of this study indicated that all strains of Lactobacillus were able to bind AFM1 molecules. This ability was profoundly dependent on the product characteristics such as fermentation condition, storage period, bacterial population, kind of culture, and viability of the bacteria. Fermentation was considered as the most effective factor on AFM1 removal, and generally the reduction rate of AFM1 in fermented products with a longer incubation or storage period was much more than the non-fermented products with a short storage period. This systematic review revealed that the heat-treated bacteria were better binder for AFM1 when compared with viable bacteria, probably in primary hours, but during a long period of time of incubation or storage, the viable bacteria were more effective. It seems that among all Lactobacillus strains, *L. acidophilus* and *L. rhamnosus* were the most effective as far as they could reduce the levels of AFM1 by 99%. However, binding ability of each strain is dependent on the experimental conditions. Consequently, application of Lactobacillus strains in production of fermented dairy products from the contaminated milk can be used as an efficient method to reduce the AFM1 level in the final product. Additional studies are needed to compare the AFM1 binding potential of different strains of Lactobacillus and also the mechanisms involved in the removal process of AFM1 by Lactobacillus strains.

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Authors' Contribution

M.Z: Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. M.S: Data curation, Writing - original draft, Writing - review & editing. A.A: Conceptualization, supervision, writing - review & editing.

Conflict of Interest

None declared.

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