



## Aflatoxin M<sub>1</sub>: biological decontamination methods in milk and cheese

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### Abstract

Dairy cattle when fed on aflatoxin B<sub>1</sub> may excrete aflatoxin M<sub>1</sub> in milk as a consequence of dietary exposure. Once AFM<sub>1</sub> is excreted in milk, it is present in dairy products such as cheese, yogurt, among others. This mycotoxin is quite resistant to temperature therefore heat treatments like pasteurization and ultra-pasteurization are not enough to inactivate it. In this context, this article provides an overview of the biological decontamination methods on milk and cheese of the last decade, as a contribution to evaluate the evolution of this strategy as well as its efficiency according to their authors. Relevant studies published between January 2009 and May 2019 were selected after a systematic search of the literature in PubMed, Science direct and Google Scholar databases. According with our research in the last decade few studies have been published on these methods and unfortunately none of the published studies tested such methods in cheese. Throughout the research many studies on decontamination methods were found, however, in phosphate buffered saline solution or in culture medium. Further studies on biological decontamination on milk and mainly on cheese are necessary for this technique to be better developed and applied to a large scale in the industries.

**Keywords:** Aflatoxin; biological decontamination; cheese; milk; systematic review.

**Practical Application:** One of the main objectives of the food industry is to avoid mycotoxin contamination in processes and products. Microbial decontamination of aflatoxin M<sub>1</sub> as the most promising strategy due to their effectiveness and environmentally friendly behavior.

### 1 Introduction

Aflatoxins are the most widely known and distributed mycotoxins in foods and feeds (Wochner et al., 2018). Aflatoxins, mainly aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) are potent carcinogenic, teratogenic and mutagenic agents. When lactating animals are fed with feed containing aflatoxins (mainly AFB<sub>1</sub>), these are biotransformed resulting in a new compound denominated aflatoxin M<sub>1</sub> (AFM<sub>1</sub>), which is soluble and excreted in the milk of these animals (Fink-Gremmels, 2008; Gonçalves et al., 2015b). According to the World Health Organization and the *International Agency for Research on Cancer* both AFB<sub>1</sub> and AFM<sub>1</sub> are classified as belonging to group 1, carcinogenic to humans (World Health Organization, 2002).

Several studies have demonstrated evidence of human exposure to aflatoxin M<sub>1</sub> due to the consumption of contaminated milk and dairy products (Bovo et al., 2013; Campagnollo et al., 2016; Corassin et al., 2013; Fernandes et al., 2012; Shundo et al., 2009; Trombete et al., 2013; Womack et al., 2016).

Once in the milk, AFM<sub>1</sub> is not degraded and can resist different industrial treatments including milk sterilization or to any other heat treatment (Campagnollo et al., 2016; Assaf et al., 2019). Milk and dairy products are frequently consumed by a portion of the population considered vulnerable, such as children and the elderly (Fallah et al., 2009; Prandini et al., 2009). For the

reasons mentioned above, AFM<sub>1</sub> contamination is a serious health problem.

Knowledge about the negative impacts of aflatoxins on the economy and health led to investigations for strategies to prevent their formation in food, as well as to eliminate, inactivate or reduce the bioavailability of these toxins in contaminated products (Corassin et al., 2013; Gonçalves et al., 2015a). Improvements in agricultural practices, antifungal agents and genetic engineering may be used as a means of prevention (González et al., 2005). Aflatoxin elimination or inactivation may be achieved by physical, chemical and biological methods (Corassin et al., 2013).

Physical and chemical methods are not the most appropriate in terms of safety, economy and product quality, either because they are not efficient at removing contaminants, or high costs and nutritional and sensory damage to the product (Stoev, 2013; Wochner et al., 2018). Thus, the biological methods emerged and gained popularity due to its friendliness to both environment and body health (Peng et al., 2018).

In this context the aim of this study was to review the biological methods of aflatoxin M<sub>1</sub> decontamination in milk and cheese published in the last decade as a contribution to evaluate the evolution of this strategy as well as its efficiency according to its authors.

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## 2 Search strategy

The bibliographic research, inclusion and exclusion criteria and data collection were performed based on the protocol Cochrane (Higgins, 2011). Relevant studies published between January 2009 to May 2019 were selected after a systematic search in the following databases PubMed, Science Direct and Google Scholar databases, using key words like: “Aflatoxin M<sub>1</sub>”, probiotics and lactic acid bacteria binding OR “Aflatoxin M<sub>1</sub> in milk”, probiotics and lactic acid bacteria binding OR “biological degradation”, aflatoxin M<sub>1</sub> in dairy products OR “biological binding”, aflatoxin M<sub>1</sub> in milk.

### 2.1 Inclusion and exclusion criteria

During first screening, the full texts of potentially eligible articles were downloaded. Then, the citations transferred were examined twice for inclusion and final eligibility criteria. The inclusion criteria were: (1) Cross-sectional descriptive data, (2) Full-text article available (3) Original research studies, (4) Decontamination method used and (5) Type of product examined (milk or cheese). In addition, in order to avoid any error in the translation process and also the clarity of data expression, based on previously published meta-analysis studies in Food Science (Cherkani-Hassani et al., 2016; Khaneghah et al., 2018), only articles published in English were included. Citations that did not meet these criteria were excluded.

### 2.2 Data collection

After screening, 405 articles were retrieved and evaluated for eligibility. 394 articles were excluded in the initial evaluation due to duplicates or based on their title and abstract content. Finally, 11 articles met the inclusion criteria and were included in the review.

## 3 Aflatoxin M<sub>1</sub> milk, cheese and decontamination methods

### 3.1 Aflatoxin M<sub>1</sub> synthesis and excretion

One of the most widely known and largely distributed mycotoxin groups in food, with proven toxic properties are aflatoxins (Campagnollo et al., 2016; Varga et al., 2015) can be produced by approximately twenty species of three different species of the genus *Aspergillus*, being *Aspergillus flavus* the main aflatoxins producer (Elsanhoty et al., 2014).

The occurrence of aflatoxins in food is unavoidable and influenced by environmental factors. The extent of its contamination is not predictable and may vary with agronomic practices and product susceptibility to fungus invasion during the pre-harvest, storage and processing stages (Araújo, 2012). *Aspergillus* spp. grows on a wide variety of substrates, and most food and feed are susceptible to invasion by aflatoxigenic strains at any stage of production, processing or storage (Oliveira & Corassin, 2014). In this context, aflatoxins can be found as natural contaminants in cereals, cereal by-products, oilseeds, cassava, and a whole set of foods for humans such as dried fruit, spices, oilseeds, milk and dairy products (Bhat et al., 2010; Gonçalves et al., 2015b).

Aflatoxins are present throughout the world, but mainly in tropical climate areas. Their appearance in agricultural products occurs in hot and humid weather conditions, and in poor or inadequate storage facilities. The most influential factors on the growth and production of aflatoxins are air humidity and temperature, being optimum humidity above 80% and the maximum toxin production temperature between 25 and 27 °C (Abbas, 2005; Spinosa et al., 2008). Aflatoxin production can be influenced by some other factors such as: substrate composition, pH, oxygen and carbon dioxide content, microbial competition, mechanical damage, contaminant fungus lineage, plant stress and use of fungicides (Hussein & Brasel, 2001; Magan & Olsen, 2006).

Nutrition is one of the most important factors in dairy production, representing the main cost in this activity. Nutrient requirements of dairy cows vary according to the stage of pregnancy and lactation. The nutrients required by dairy cows are energy, fiber, protein, water, vitamins and minerals. Pasture provides a balanced source of feed, however is not enough to maintain high-producing dairy cows. Energy is the decisive factor in milk production: it determines milk yield and composition, as well as body weight. The main energy sources in dairy cow feed are carbohydrates and fiber (corn silage, ground corn, whole cottonseed, high moisture shelled corn, corn gluten meal, soybean hulls and meal). Protein and fats in feeds can also be used as energy sources (Gonçalves et al., 2015b).

Dairy cattle feed may be naturally and simultaneously contaminated by several fungi that are able to produce different toxins. According to Alonso et al. (2013) most of the silage is made up from annual crops. Therefore, mycotoxin concentration in the feed may vary annually. Several species in the genera *Aspergillus*, *Penicillium*, *Alternaria*, *Trichoderma*, *Claviceps* and others make up the storage microbiota and are responsible for the production of mycotoxins found in grains and forage (Gonçalves et al., 2015b).

Dairy cattle are highly exposed to the action of mycotoxins due to their dependence on stored and bulky concentrates such as hay or silage (Jobim et al., 2001). Ruminants are less susceptible to the effects of mycotoxins because the rumen to be populated by microorganisms (*fungi, bacteria, protozoa, archaea*). Among these, protozoans are the major microorganisms responsible for mycotoxin detoxification (Gonçalves et al., 2015b). This process, however, is not always efficient and some toxic metabolites can be excreted in the milk (European Food Safety Authority, 2004; Jouany & Diaz, 2005).

When dairy cattle ingest feed containing aflatoxin (mainly AFB<sub>1</sub>), part of it is degraded by rumen microorganisms, resulting in the metabolite aflatoxicol (Kuilman et al., 1998). The remaining non-degraded fraction is absorbed by passive diffusion (Fink-Gremmels, 2008) and metabolized in the liver where the biotransformation to AFM<sub>1</sub> occurs being excreted in milk and urine (Murphy et al., 2006; Oatley et al., 2000).

### 3.2 Prevalence of aflatoxin M<sub>1</sub> in milk and cheese

Milk is the product of complete and uninterrupted milking under healthy conditions of healthy, well fed and rested cows. It is the first food consumed by man and one of the most complete.

Milk has essential elements, micronutrients, amino acids and fatty acids in larger portions than in any other product. In addition, it contains high quality proteins, high percentage of calcium and some bioactive substances like enzymes, hormones and cytokines (Food and Agriculture Organization, 2019).

Cheese is one of the oldest foods recorded in the entire history of humanity. The Egyptians are among the first people to domesticate goats and sheep, being also one of the first to use milk and cheese as a source of food (Cruz et al., 2017). The primitive cheese was only coagulated milk, devoid of whey and salty. In the nineteenth century the great boom occurred in the consumption of cheese, after all, its production switched from handmade industrial scale, and the process of pasteurization is fundamental for this shift (Chalita et al., 2009). Throughout the ages, cheese has evolved to what is known today and the great typological variation of cheeses is related to the adaptation of this product to the different tastes and cultural customs (Instituto Estadual do Patrimônio Histórico e Artístico de Minas Gerais, 2012).

The occurrence of aflatoxin  $M_1$  in cheese produced from milk contaminated with these toxin is a reality already described by several authors (Armorini et al., 2016; Campagnollo et al., 2016; Fernandes et al., 2012; Hassan et al., 2018; Iha et al., 2011; Kav et al., 2011; Koluacik et al., 2015; Manetta et al., 2009; Shahbazi et al., 2017; Xiong et al., 2018; Yilmaz & Altinci, 2019; Yoon et al., 2016; Zheng et al., 2017). In relation to the presence of AFM<sub>1</sub> in cheeses others factors, further to the presence of toxin in milk should be considered, as: kind of cheese, processing steps, kind of milk used to produce (raw or pasteurized), if there is period of maturation, addition of herbs among others (Campagnollo et al., 2016).

Concerns with the negative impacts of aflatoxins on health and economics has led to the investigation of strategies to prevent their formation in food (mainly feed), as well as to eliminate, inactivate or reduce the availability of these mycotoxins in contaminated products (Bovo et al., 2010; Corassin et al., 2013; Gonçalves et al., 2015a, 2017).

Contamination may be prevented by good agricultural practices, antifungal agents, genetic engineering and storage control. Mycotoxin elimination or inactivation may be achieved by physical, chemical and biological methods (Corassin et al., 2013; Trombete et al., 2013). However, since these foods are already contaminated, adsorbents can be used in the animal feed, in order to reduce the bioavailability of these mycotoxins and thus avoid the excretion of AFM<sub>1</sub> in milk, as described by (Firmin et al., 2011; Gonçalves et al., 2017).

If the fore-mentioned methods are not applied, and AFM<sub>1</sub> is present in milk, according to Fallah (2010), Manetta et al. (2009) and Trombete et al. (2013), it will be also present in its dairy products (cheese, yogurts, etc.). According to the same authors and Campagnollo et al. (2016) this occurs because AFM<sub>1</sub> is stable at the various stages of processing for the manufacture of dairy products such as pasteurization, UHT, milk coagulation, acidification, etc. In addition Ardic et al. (2009) emphasized that cheese is a potential source of AFM<sub>1</sub> when compared to other dairy products, because AFM<sub>1</sub> is associated with casein, which is highly concentrated during its production.

### 3.3 Biological methods for decontamination

Several methods have been used to reduce the availability of mycotoxins, especially aflatoxins, in food intended for humans. These methods may be physical, chemical or biological (Bovo et al., 2010).

The main physical methods used to reduce the availability of mycotoxins are thermal inactivation, ultraviolet light, ionizing radiation, or extraction with solvents. Chemical methods include chlorination and oxidant or hydrolytic agents (El-Nezami et al., 1998; Oliveira & Corassin, 2014). However, both chemical and physical methods have advantages and disadvantages, as they do not completely remove completely the toxin, are expensive and cause nutritional and organoleptic losses to the products (Magan & Olsen, 2006). Therefore, the use of microorganisms to degrade mycotoxins has become an attractive alternative for control or reducing the bioavailability of these toxins in food (Corassin et al., 2013; Fazeli et al., 2009; Gonçalves et al., 2015b).

Biological methods are based on the action of microorganisms such as yeasts, molds, bacteria and algae on mycotoxins, through competition for nutrients and space, interaction and antibiosis (Fazeli et al., 2009). In addition, these decontamination methods have been well studied since they are efficient, cost-effective, and in many cases are already used in food production, such as acid lactic bacteria and yeast *Saccharomyces cerevisiae* (Corassin et al., 2013; Gonçalves et al., 2015a).

According to the criteria of inclusion and exclusion of the research, eleven articles, published in the last decade, on biological methods of decontamination in milk and cheese, shown in Table 1, were selected.

## 4 Challenges and implications of aflatoxin $M_1$ methods of decontamination in milk and cheese

Although the occurrence of aflatoxins in milk and dairy products has been frequently reported, there is little information about biological decontaminating in these products. In the last decade, some studies around the world validated microbial decontamination of aflatoxin  $M_1$  as the most promising strategy due to their effectiveness, specificity and environmental friendly behavior to reduce or eliminate possible contamination by aflatoxins (Ismail et al., 2016).

The main concern with exposure to aflatoxin  $M_1$  and consumption of dairy products is the exposure of children and the elderly (Fallah, 2010). Decontamination procedures may be physical, chemical and biological, should not modify the nutritional or technological milk and dairy products properties and be affordable and easy to use (Lee et al., 2015). Physical and chemical methods are not the most appropriate regarding safety, economy and the quality of treated products high costs or they result in nutritional and sensory damage to the product (Wochner et al., 2018). In this context the proposal of biological methods of decontamination, aflatoxins or mycotoxins in general, is to improve the quality and safety of the products.

The application of biological methods for decontamination of aflatoxins has been noticeably in recent years, resulting in new data as the studies reported in Table 1.

**Table 1.** Biological methods of decontamination in milk in different countries in the last 10 years (2009 to date).

Kind of Sample analyzed	Initial and final concentration of toxin ( $\mu\text{g/L}^1$ or $\mu\text{g/kg}^2$ )	Decontamination range (%)	Contact time (minutes)	Biological method and concentration	Reference
Milk	0.08-0 <sup>1</sup>	100	40	<i>Saccharomyces cerevisiae</i> immobilized with perlite	Foroughi et al. (2019)
	0.23-0.043 <sup>1</sup>	81.3	80	10 <sup>8</sup> CFU/mL	
Skim Milk	150-121.8 <sup>1</sup>	18.8	960	<i>Lactobacillus rhamnosus</i> 10 <sup>10</sup> CFU/mL	Abdallah et al. (2018)
	150-110.1 <sup>1</sup>	26.6		<i>Lactobacillus rhamnosus</i> heat-treated 10 <sup>10</sup> CFU/mL	
Skim milk	50-9.72 <sup>1</sup>	80.5	720		Abdelmotilib et al. (2018)
	50-6.68 <sup>1</sup>	86.6	1440	Pool of microorganisms <sup>c</sup> heat-treated	
	50-5.70 <sup>1</sup>	88.6	2880	10 <sup>9</sup> CFU/mL	
Milk	50-4.56 <sup>1</sup>	90.9	4320		Kuharić et al. (2018)
	0.05-0.0028 <sup>2</sup>	94.5	120	<i>Lactobacillus plantarum</i> heat-treated 10 <sup>8</sup> CFU/mL <i>Lactobacillus helveticus</i> 10 <sup>10</sup> CFU/mL	
Milk	0.05-0 <sup>1</sup>	100	60	Pool of microorganisms <sup>a</sup> <i>Saccharomyces cerevisiae</i> 10 <sup>10</sup> CFU/mL	Ismail et al. (2017)
	0.01- 0.0008 <sup>1</sup>	92.0		<i>Saccharomyces cerevisiae</i> 10 <sup>10</sup> CFU/mL	
	0.01-0.0013 <sup>1</sup>	87.0		Pool of microorganisms <sup>a</sup>	
	0.050-0.030	30.9	1440	<i>Lactobacillus acidophilus</i>	
Milk	0.050-0.010	78.3	2880	10 <sup>8</sup> CFU/mL	El-Kest et al. (2016)
	0.050-0.022	54.8	1440	<i>Bifidobacterium lactis</i>	
	0.050-0.019	62.5	2880	10 <sup>8</sup> CFU/mL	
	0.050-0.004	91.4	1440	<i>Lactobacillus acidophilus</i> and <i>Bifidobacterium lactis</i>	
	0.050-0.002	96.2	2880	10 <sup>6</sup> CFU/mL	
Skim milk	200-61.8	69.1	360	<i>Lactobacillus plantarum</i>	Abbès et al. (2013)
	200-46.2	76.9	1440	10 <sup>8</sup> CFU/mL	
	200-28.2	85.9	360	<i>Lactobacillus rhamnosus</i>	
	200-9.8	95.1	1440	10 <sup>8</sup> CFU/mL	
	0.5-0.048 <sup>1</sup>	90.3	30	<i>Saccharomyces cerevisiae</i> heat-treated	
UHT skim milk	0.5-0.037 <sup>1</sup>	92.7	60	10 <sup>9</sup> CFU/mL	Corassin et al. (2013)
	0.5-0.443 <sup>1</sup>	11.5	30	Pool of lactic acid bacteria <sup>b</sup>	
	0.5-0.441	11.7	60	heat-treated 10 <sup>10</sup> CFU/mL	
	0.5-0 <sup>1</sup>	100	60	<i>Saccharomyces cerevisiae</i> and Pool of lactic acid bacteria <sup>b</sup> 10 <sup>10</sup> CFU/mL	
	10-5.48 <sup>1</sup>	45.17		<i>Bifidobacterium bifidum</i> 10 <sup>9</sup> CFU/mL	
Milk	10-6.78 <sup>1</sup>	32.20		<i>Lactobacillus johnsonii</i> 10 <sup>9</sup> CFU/mL	Serrano-Niño et al. (2013)
	10-7.35 <sup>1</sup>	26.50	180	<i>Lactobacillus reuteri</i> 10 <sup>9</sup> CFU/mL	
	10-7.55 <sup>1</sup>	24.54		<i>Lactobacillus rhamnosus</i> 10 <sup>10</sup> CFU/mL	
	0.5-0.31	37.75		<i>Bifidobacterium lactis</i> heat-treated 10 <sup>10</sup> CFU/mL	
UHT skimmed milk	0.5-0.33	32.24	15	<i>Lactobacillus bulgaricus</i> heat-treated 10 <sup>10</sup> CFU/mL	Bovo et al. (2013)
	0.5-0.37	24.46		<i>Lactobacillus bulgaricus</i> heat-treated 10 <sup>10</sup> CFU/mL	
Skim milk	50-26.9 <sup>1</sup>	46.1	120	<i>Lactobacillus bulgaricus</i>	El Khoury et al. (2011)
	50-20.8 <sup>1</sup>	58.5	360	10 <sup>6</sup> CFU/mL	
	50-38.7 <sup>1</sup>	22.6	120	<i>Streptococcus thermophilus</i>	
	50-31.2 <sup>1</sup>	37.7	360	10 <sup>6</sup> CFU/mL	

<sup>1</sup> $\mu\text{g/L}$ ; <sup>2</sup> $\mu\text{g/kg}$ ; <sup>a</sup>*Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactococcus lactis* and *Saccharomyces cerevisiae* (2:1:1:1); <sup>b</sup>*Lactobacillus rhamnosus*, *Lactobacillus delbrueckii* spp. *Bulgaricus* and *Bifidobacterium lactis*; <sup>c</sup>*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium bifidum*, *Saccharomyces cerevisiae* and *Kluyveromyces fragilis*.



Foroughi et al. (2019) tested the decontamination of milk samples contaminated with different concentrations of AFM<sub>1</sub> (0.08; 0.13; 0.18; 0.23 µg/L) using *S. cerevisiae* 10<sup>8</sup> (CFU/mL) immobilized with perlite. The milk samples were passed through the biofilter at three different treatment times (20, 40 and 80 minutes). For the milk sample with a lower concentration of AFM<sub>1</sub> (0.08 µg/L) 40 minutes treatment was sufficient to eliminate all the toxin. However, in the sample with the highest concentration (0.23 µg/L), the best result 81.3% was obtained only at the highest circulation time. According to the authors, the amount of AFM<sub>1</sub> removed is related to the initial concentration of toxin and the time of circulation, in general the longer the treatment, the lower concentration of AFM<sub>1</sub> residue is present in milk. In addition, the authors report that the processing influenced the microbial load of the milk, which increased linearly with the increase of the circulation time ( $P < 0.05$ ); concluding that the shortest circulation time would be the most desirable. Despite the increase in microbial load, the physicochemical properties of the milk did not change. The authors conclude that the use of *S. cerevisiae* immobilized with perlite may be a practical solution to address the problem of contamination of aflatoxins in dairy products.

Kuharić et al. (2018) selected, isolated and identified ten species of lactic acid bacteria from milk and dairy products with the objective to select a strain which binds more effectively to AFM<sub>1</sub>. In addition, they assessed whether the heat treatment of LAB would affect binding ability. For this, the authors selected 10 species of LAB, which could be used viable or treated at the concentration of 10<sup>8</sup> (CFU/mL). These LABs were added to milk containing AFM<sub>1</sub> (0.05 µg/L) and the binding capacity was evaluated after 2, 4 and 24 hours. The authors reported that binding efficiency ranged from 21 to 92% for viable cells and from 26 to 94.5 for thermally treated cells. According to Kuharić et al. (2018) although the mechanisms of action of lactic acid bacteria on aflatoxins has not yet been fully elucidated, some studies suggest that AFM<sub>1</sub> molecules bind physically to cell wall components, mainly polysaccharides and peptidoglycans, instead of creating covalent bonds with or being metabolized by lactic acid bacteria. In addition, they emphasize that the heat treatment seems to increase the binding surface for AFM<sub>1</sub>, providing not only the wall of the lactic acid bacteria cell, but also other components. Finally, these authors conclude that the binding of AFM<sub>1</sub>-LAB is reversible, and that the percentage of reversibility is greater in untreated cells.

Ismail et al. (2017) evaluated three strains of lactic acid bacteria, one strain of *S. cerevisiae* and a mixture of all, in relation to their ability to reduce AFM<sub>1</sub> in milk. Samples were contaminated with different concentrations of AFM<sub>1</sub> (0.05 and 0.01 µg/L) and four different concentrations of microorganisms were tested (10<sup>7</sup>, 10<sup>8</sup>, 10<sup>9</sup>, 10<sup>10</sup> CFU/mL). The authors reported that significant differences were observed in relation to the binding capacity of the different concentrations of microorganisms in the different concentrations of AFM<sub>1</sub>. They observed that the binding capacity of each microorganism is directly proportional to its concentration, i.e., maximum binding capacity was recorded at 10<sup>10</sup> CFU/mL and minimum at 10<sup>7</sup> CFU/mL. The authors also reported that the general trend of binding by microorganisms was found in the following order *S. cerevisiae* > pool of microorganisms

> *Lactobacillus helveticus* > *Lactobacillus plantarum* > *Lactococcus lactis*. Finally, the authors concluded that the 10<sup>10</sup> CFU/mL concentration can be used efficiently to reduce AFM<sub>1</sub> levels to the limit considered safe by legislation; the AFM<sub>1</sub>-LAB binding is a reversible process, since lavage of the complex results in the release of AFM<sub>1</sub>. Similarly, (Corassin et al., 2013) evaluated the ability of *S. cerevisiae* and pool of three different lactic acid bacteria (*Lactobacillus rhamnosus*, *Lactobacillus delbrueckii* spp. *bulgaricus* e *Bifidobacterium lactis*) alone or in combination to bind AFM<sub>1</sub> in UHT milk skim spiked 0.5 µg AFM<sub>1</sub>/L. All the lactic acid bacteria (10<sup>10</sup> CFU/mL) and *S. cerevisiae* (10<sup>9</sup> CFU/mL) cells were heat killed (100 °C, 60 minutes) and then used for checking the effect of contact time (30 or 60 minutes). These authors reported that *S. cerevisiae* showed higher capability to bind AFM<sub>1</sub> in milk than lactic acid bacteria pool and when using *S. cerevisiae* and lactic acid bacteria pool associated a significant increase observed in the percentage of AFM<sub>1</sub> bound. In agreement Abdelmotilib et al. (2018) reported that lactic acid bacteria and yeast strains can detox AFM<sub>1</sub> in contaminated milk. However, a combination of LABs and yeasts could be better for removal and elimination of AFM<sub>1</sub> from milk. Furthermore, these authors emphasize that bacteria and yeasts could be used as food additives to reduce the bioavailability of the aflatoxins in dairy products.

El Khoury et al. (2011) reported that when lactic acid bacteria cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) in PBS and skimmed processing milk were compared, the binding was much greater in milk. According to these authors the main reason of that is may be due to the binding properties of AFM<sub>1</sub> to milk casein. Furthermore, they emphasized that binding level of AFM<sub>1</sub> by *L. bulgaricus* increased with time and as they expected *S. thermophilus* showed a lower binding capacity to removal AFM<sub>1</sub> making in comparison to *L. bulgaricus*. In conclusion the authors reported that LAB seem to play a crucial role in AFM<sub>1</sub> removal.

Bovo et al. (2013) compared treatments for binding AFM<sub>1</sub> in skimmed milk in two different temperatures 4 and 37 °C (last T°C the results are show in Table 1. The binding percentage of AFM<sub>1</sub> in UHT skimmed milk at 4 °C was 13.51, 19.7 and 37.75 for *L. bulgaricus*, *L. rhamnosus* and *B. lactis*, respectively. These Authors reported that the temperature is no significant difference for *L. rhamnosus* and *B. lactis*, only for *L. bulgaricus* at 37 °C. In addition, Bovo et al. (2013) emphasizes that the LAB/AFM<sub>1</sub> complex was unstable and the amount of toxin released to the solution varied widely from strain to strain. Corroborating with this Serrano-Niño et al. (2013) reported that AFM<sub>1</sub> binding capacity was reversible process since all strain tested released a small portion of bound AFM<sub>1</sub> after a single wash with PBS. Furthermore, these authors emphasized that variations in binding abilities among strains suggest that different binding sites could be present in different strain and there could be differences between sites in each bacterium.

## 5 Conclusions

Worldwide contamination of milk and cheese with aflatoxin M<sub>1</sub> has been raised as a crucial issue, which becomes more critical mainly due to potential dietary exposure of children and

the elderly. Some studies around the world validated microbial decontamination of aflatoxin M<sub>1</sub> as the most promising strategy due to their effectiveness and environmentally friendly behavior.

Our study reviewed biological methods for decontamination of aflatoxin M<sub>1</sub> in milk and cheese, as well as challenges and implications on these methods. We observed that in the last decade few studies have been published on these methods and unfortunately none of the published studies tested such methods in cheese. Throughout the research were found many studies on decontamination methods however in phosphate buffered saline solution or in culture medium. Further studies on biological methods for decontamination in milk and mainly in cheeses and dairy products are necessary for this technique to be better developed and applied to large scale industries.

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