Effect of production and storage of probiotic yogurt on aflatoxin M_1 residue

H. Montaseri [1] (PhD), S. Arjmandtalab [2*] (MSc), G. Dehghanzadeh [2] (Pharm D), S. Karami [2] (MSc),
M.M. Razmjoo [2] (Pharm D), M. Sayadi [3] (MSc), A. Oryan [4] (PhD)

1. Department of Pharmaceutics, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
2. Deputy of Food and Drug Control, Shiraz University of Medical Sciences, Shiraz, Iran
3. Behbahan Faculty of Medical Sciences, Behbahan , Iran
4. Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

ABSTRACT

Introduction: Aflatoxin M_1 is an important mycotoxin frequently found in milk and dairy products. The main objective of this work was to study the stability of AFM_1 during production and refrigerated storage of probiotic yogurt.

Materials and methods: Two kinds of probiotic yogurt were made by cow's milk artificially contaminated with aflatoxin M_1 at a level of 100 ng/l, and fermented to reach pH 4.5. The yogurts were stored at 4 °C for up to 21 days. Analysis of aflatoxin M_1 in yogurt was carried out, using immunoaffinity column extraction and liquid chromatography coupled with fluorometric detection.

Results: The aflatoxin M_1 levels in the probiotic yogurts showed a significant decrease ($p<0.05$) compared with those initially added to milk. During the refrigerated storage the aflatoxin M_1 was lower in 'DELVO-YOG MY 1821'(MY 1821) yogurt than 'FD-DVS ABY3' (ABY3), but the difference was not significant ($p>0.05$). The percentage loss of the initial amount of aflatoxin M_1 in milk was estimated at about 41% and 49% by the end of storage for yogurts made by ABY3 and MY1821 yogurt, respectively. Loss of viability of the probiotic bacteria in presence of aflatoxin M_1 was strain dependent. Aflatoxin M_1 had no remarkable effect on viability of tested bacteria.

Conclusion: The probiotic yogurt can reduce the AFM_1 content of initial milk during production and storage. More studies are needed to investigate the effectiveness of other mixed probiotic cultures with different composition, to reduce the AFM_1 content of milk.

Introduction

Aflatoxins are major classes of mycotoxins produced by some *Aspergillus* species (*A. flavus*, *A. parasiticus* and rarely *A. nomius*) that occur in a wide variety of commodities including cottonseed, peanuts, tree nuts, spices, dried fruits and cereals (especially maize) during growth, harvest, post-harvest and storage (Pitt, 2000). There are currently 20 similar compounds described by the term aflatoxin (Prandini et al., 2009), but the most prevalent and toxic one is aflatoxin B_1 (AFB_1) (Creppy, 2002).

Aflatoxin M_1 (AFM_1), the 4-hydroxy metabolite of AFB_1, is the predominant metabolite of AFB_1 and can be found in milk and milk products obtained from lactating animals ingesting

feed contaminated with AFB_1 (Prandini et al., 2009). The conversion rate of the ingested AFB_1 to AFM_1 varies from 0.5% to 5% for lactating animals (Neal et al., 1998). Acute AFM_1 toxicity is similar or slightly milder than that of AFB_1 and its carcinogenic potential is about ten times less than that of AFB1 (FAO, 2001), but its cytotoxic and carcinogenic effects have been demonstrated in several species (Murphy et al., 2006).

The International Agency for Research on Cancer (2002) has classified AFM_1 as belonging to Group 1, a human carcinogen. The high intake of dairy products by human population, especially by infants and young children (Neal et al., 1998) and the toxic effects of AFM_1, led to an increased concern about the establishment of measures to control AFM_1 contamination. The European Commission Regulation set a maximum permissible limit of 0.05 µg/kg for AFM_1 in raw milk, heat-treated milk and milk for the manufacture of milk-based products (European Commission, 2006a).

---

*Corresponding author
Email: soliman.arjomand@yahoo.com

There are many reports on AFM_1 contamination in milk and dairy products (Iha et al., 2013) and in some regions such as Iran the prevalance is high (Fallah, 2010a; Fallah, 2010b; Fallah et al., 2011; Ghazani, 2009; Heshmati and Milani, 2010). When dairy products are manufactured from the AFM_1 contaminated milk, the toxin could be detected in them (Bakirci, 2001). Unfortunately, the content of AFM_1 is relatively stable during normal processing and storage of various dairy products (Fallah, 2010a; Iha et al., 2013), and currently there are no acceptable methods to counteract the AFM_1 occurrence in milk and dairy products (El Khoury et al., 2011). Thus, a practical and effective method is needed to be developed for the detoxification of AFM_1 in milk and dairy products and implemented in dairy industry especially in the countries with high level of milk contamination.

According to the current adopted dentition by FAO (2001), "probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". A number of health benefits are claimed in favor of products containing probiotic organisms including antimicrobial activity and gastrointestinal infections, improvement in lactose metabolism, antimutagenic properties, anticarcinogenic properties, reduction in serum cholesterol, anti-diarrhoeal properties, immune system stimulation, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection (Kurmann and Rasic, 1991; Shah, 2000, 2001). The main species believed to have probiotic characteristics are *Lactobacillus acidophilus*, *Bifidobacterium spp.*, and *L. casei* (Shah, 2007). Yogurt, the best carrier of probiotics, traditionally is manufactured using *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus* (*L. bulgaricus*) as starter cultures (Lourens-Hattingh and Viljoen, 2001). For this product to be considered as a probiotic, one or more species of, *L. acidophilus*, *Bifidobacterium* and *L. casei* are incorporated as dietary adjuncts. The longer incubation period required and poorer resultant product quality, are the two main factors that preclude such practice commercially. Thus, the normal practice is to make the product with two starter organisms, e.g., *Str. thermophilus* and *L. bulgaricus*, and one or more species of probiotic bacteria (Shah, 2007). The viability of probiotic microorganisms in the final product is the most important qualitative parameter of probiotic products as it determines their pharmaceutical effectiveness. Loss of viability of probiotics during the fermentation process and refrigerated storage is a major issue in the production of probiotic yogurt (Mortazavian et al., 2007). Minimum viability of the probiotic bacteria must be above the 10^6 cfu/gr in the probiotic products (Lourens-Hattingh and Viljoen, 2001).

The best way to control the presence of aflatoxin in food and feeds is to prevent their formation, but this is not always possible, so various physical and chemical methods have been used to detoxify these toxins from food and feed materials; safety issues, possible loss in nutritional quality of food, limited efficacy and cost implications of these method, have led to searching for alternative methods. Recently there has been an increased interest in the use of microorganisms such as bacteria, yeast and fungi to reduce the toxic effect of mycotoxins (Kabak et al., 2006).

Different studies have demonstrated the potential of probiotic bacteria to remove and reduce bioaccessibility of AFM_1 in milk and liquid media, using *in vitro* and *in vivo* model systems (Bovo et al., 2012; Corassin et al., 2013; Kabak and Ozbey, 2012; Serrano-Niño et al., 2013). On the other hand, studies on the stability of AFM_1 in yogurt are limited and contradict each other. Studies on the influence of AFM_1 on yogurt microorganisms are also very limited (Govaris et al., 2002).

To the authors knowledge there is no data in the literature regarding the stability of AFM_1 and the effect of AFM_1 on the viability and survival of probiotic bacteria during production and storage of the probiotic yogurt.

Thus, the main objective of this work was to study the stability of AFM_1 during production and refrigerated storage of probiotic yogurt. In addition, the effect of AFM_1 on the viability of probiotic strains of the starter cultures was also investigated.

Materials and methods

Standard preparation of AFM_1

AFM_1 standard solution at concentration of 100 ng/ml, was supplied from Sigma Chemical Co., USA. The working solutions were prepared in appropriate dilution by acetonitrile. Appropriate portions of the standard solution of AFM_1 were evaporated and diluted with mobile phase to obtain final concentration in the range of 0.02 to 5 ng/ml. For AFM_1 spiking solutions in the recovery study, appropriate portions of the solution of AFM_1 were evaporated and diluted to give concentrations of 0.02, 0.1 and 0.5 ng/ml.

Sample preparation and extraction

The yogurt samples were shaken manually for 2 min before being opened to ensure that the mixtures were homogeneous. The test samples (10 gr) were mixed with extract solvent methanol: water (55:45 v/v). After shaking for 10 min, the mixture was centrifuged (Rotafix 32A, Hettich, Germany) at $4000 \times g$ for 25 min (2 or 3 times). The upper oil layer was discarded. The supernatant (30 ml) was placed into a 125 ml Erlenmeyer flask and 60 ml water was added. The mixture was passed through Whatman no.1 filter paper. Approximately 60 ml filtrate was collected and proceded immediately with IAC chromatography.

Purification and isolation by immunoaffinity column

Test solutions introduced in the previous section were passed through an immunoaffinity column (IAC), Afla Star Fit 3 (Romer Labs, Tulln, Austria), at a slow steady volume (with flow rate 1 ml/min). The column was secured on a vacuum manifold and had previously been conditioned with 5 ml of phosphate-buffered saline. The column was then washed twice with 10 ml ultrapure water and the AFM_1 was eluted from the column with acetonitrile. The eluate was evaporated under nitrogen flow (Dubnof Bath BSD/D) to dryness. The residue was redissolved in mobile phase and collected in HPLC vials (Supleco, Bellefonte, PA, USA) and injected to HPLC system according to following section.

HPLC analysis

The HPLC set up was done based on the following procedure:
1. Column: Reverse phase ODS_2 – 5 μm, 250 m× 4.6 m C18 Column TSK – GEL® (TosoHas)
2. Guard Column: Guard Column NovaPak® C18 Waters
3. Mobile phase: Acetonitrile: Methanol: H2O (20:20:60)
4. Flow rate: 1 ml/min
5. Injection volume: 20 μl
6. Fluorescence detector: Waters 2475 fluorescence detector, excitation 360 nm, emission 440 nm
7. Gain: 10
8. EUFS: 1000
9. Retention time: 19.1 min.
10. HPLC System: Waters Breeze 1525 HPLC Pump, Waters 1525 Binary HPLC Pump, Waters Column Heater, Waters Bus SAT/IN, Waters Bus Lace, Waters Breeze Software

Validation of HPLC analytical method

The validation of the analytical method was based on the following criteria: selectivity, linearity, sensitivity, accuracy and precision. The selectivity of the method was evaluated by analyzing the blank and spiked samples of yogurt at levels of 0.02, 0.05, 0.1, 0.25, 0.5, 1 and 5 μg/kg. The linearity was assessed by constructing five-point calibration curve over the concentration range of 0.02-5 μg/ kg, each concentration was injected four times. The linearity was evaluated by linear regression analysis using the least squares method and expressed as correlation coefficient (R^2). The precision of the method was expressed by the limits of detection (LOD) and quantification (LOQ). The LOD, as the lowest concentration of the AFM_1 that can be clearly detected above the baseline, was determined by triplicate analysis of the spiked uncontaminated yogurts. LOQ defined as the lowest concentration of analyte that can be determined with acceptable RSD in within and between-run assays. To assess the accuracy (recovery), the blank yogurt

samples were spiked with appropriate amounts of AFM_1 working standards to obtain final concentrations of 0.02, 0.05 and 0.2 μg/kg. The recovery values were calculated by the analysis of three spiked samples with HPLC after extraction and the IAC clean-up described previously. The precision of the method was calculated in terms of within-run and between-run expressed as %RSD associated with the accuracy experiment on the same day (n=3) and on three consequent days (n=9) at the respective spiking levels.

Stability study of AFM_1 in the probiotic yogurts

The direct-in-vat-set (DVS) pouches of commercial lyophilized cultures were supplied by different starter culture suppliers. Fifty-unit pouches of commercial lyophilized culture ABY3 (containing *L. acidophilus, B .lactis*, *Str. thermophilus* and *L. bulgaricus*) was supplied by Chr-Hansen company (Horsholm, Denmark). Five-unit pouches of commercial lyophilized MY 1821 culture (containing *L. acidophilus* , *B. lactis* , *L. casei* , *Str. thermophilus* and *L. bulgaricus*) was supplied by DSM (Sydney, NSW, Australia), These cultures are currently used by the dairy industry to produce yogurt all over the world. The cultures were maintained according to the manufacturer's instructions at -18 °C. According to the manufacturers recommended procedure, a 50-unit pouch of ABY3 and a five- unit pouch of MY 1821 starter cultures were dissolved in 1 L sterilized milk separately. Four ml of ABY3 and 1 ml of MY 1821 activated cultures were used to inoculate 1 L yogurt mixture prepared as indicated below.

The non-fat skim milk powder was purchased from a local market and was reconstituted by the sterilized potable water for standardization of milk (12% milk solid-non-fat content). The AFM_1 content of the reconstitute milk was under the limit of detection (0.01 ng/ml). After heat treatment (90 ℃, 15 min), the milk was cooled down to 37 ℃ (incubation temperature). One ml of AFM_1 standard solution (100 ng/ml) was transferred to a sterile Erlenmeyer, evaporated to dryness under nitrogen flow, then 1 L of reconstituted heat-treated milk that had been cooled down to the fermentation temperature (37 °C) was added and inoculated with appropriate volume of activated starter culture according to 2.6.5. After aseptically distributing in sterile 100-ml bottles, the incubation was carried out up to pH 4.50 ± 0.02. At the end of the fermentation stage, when the fermentation was stopped, it was quickly cooled in an ice bath and stored at 4°C for 21 days. Microbiological and AFM_1 analyses were performed throughout the refrigerated storage period at 7-day intervals. The same procedure was done for the control sample without AFM_1. All the experiments carried out in triplicate.

MRS-bile agar medium (MRS agar: Merck, Darmstadt, Germany and bile: Sigma–Aldrich, Inc., Reyde, USA) was used for the selective enumeration of *L. acidophilus*, *B. lactis* and *L.casei* in the culture composition according to Mortazavian et

al. (2007), by applying the subtractive enumeration method (SEM). The plates were incubated at 37 °C for 3 days under aerobiosis and anaerobiosis. Anaerobiotic conditions were produced using the Gas Pac system (Merck, Darmstadt, Germany).

Statistical analysis

Statistical analyses were performed by Student's t-test and ANOVA with repeated measures, using the SPSS 18.0 software package program. *P* values of <0.05 were considered as significant.

Results

Validation of the methods

Selectivity of the method was assured, using immunoaffinity column for clean-up and a very selective fluorescence detector.

To assess the selectivity, the blank and spiked samples of yogurt were analyzed according to the previously described methods and the corresponding chromatograms were compared. No interfering peaks were observed at the retention time of AFM_1 (19.1) (Fig. 1). The calibration curve was linear over the concentration range of 0.02-5 µg/kg, with satisfactory coefficient of determination ($R^2 = 1$). The LOD and LOQ values, accuracy and precision of analytical method for AFM_1 yogurt are summarized in Table 1. The LODs, defined as the lowest concentration of AFM_1 that can be clearly detected above the baseline signal, was 0.01 µg/kg. The LOQs, defined as the lowest concentration of analyte that can be determined with acceptable precision and accuracy was 0.02 µg/kg. The within-run and between-run precisions were satisfactory, with RSD values always lower than 12%. The recovery values (within the range 80-110% for concentration of 0.02 µg/kg, and 83-106 % for concentration above 0.05 µg/kg) meet the requirements of the Commission Regulation (EC, 2006b).
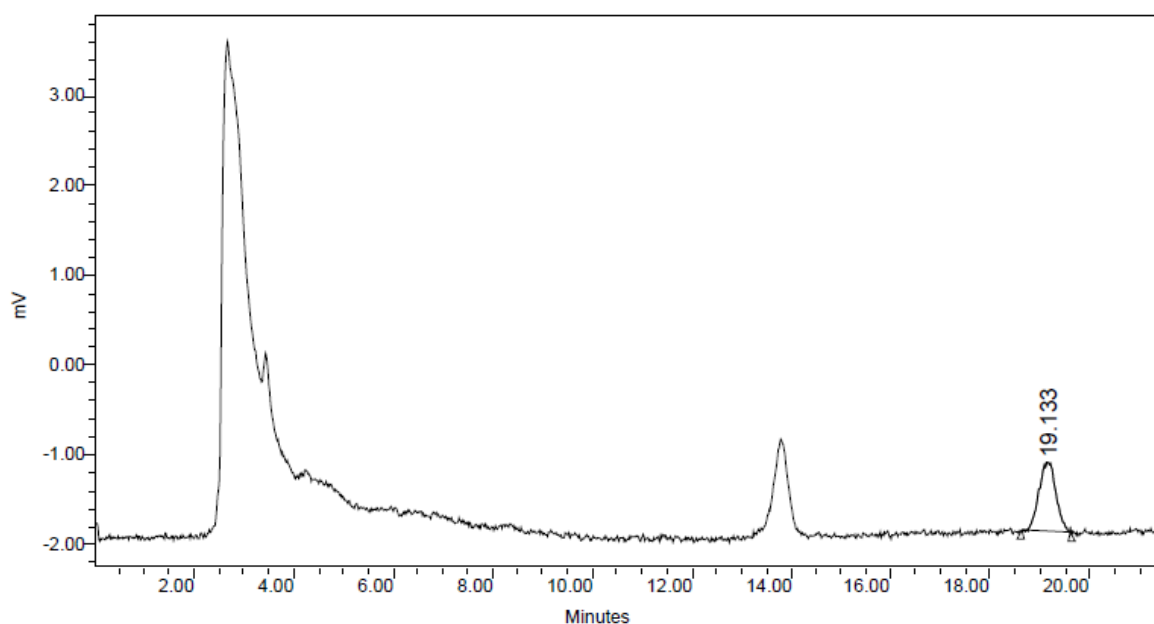

**Fig. 1**: Chromatogram for sample of milk artificially contaminated with 0.1 µg/l AFM_1

Table 1: The accuracy, precision, LOD and LOQ for AFM_1 in the yogurt samples

Spiking level (µg/kg)	Within-run (n=3)				Between-run (n=9)			
	Mean	SD	RSD (%)	Recovery (%)	Mean	SD	RSD (%)	Recovery (%)
0.02	0.021	0.002	9.1	110	0.016	0.0008	5.1	80
0.05	0.053	0.005	9.2	106	0.053	0.003	5.7	106
0.20	0.21	0.015	7.4	105	0.21	0.02	9.5	105

Table 2: Viable cell counts[1] (log cfu/gr) of probiotics (*Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus casei)* in ABY3 and MY 1821 yogurt during 21 days of refrigerated storage at 7-day intervals

Bacteria	Yogurt type	Day 1	Day 7	Day 14	Day 21	p^2 value
L. acidophilus	ABY3- 1[3]	7.43±0.09	7.26±0.05	6.6±0.1	6.45±0.11	0.015
	ABY3- 2	7.3±0.05	7.1±0.05	6.3±0.05	6.3±0.05	0.001
	p value	0.094	0.019	0.01	0.089	
	MY 1821-1	8.34±0.04	7.69±0.04	7.5±0.04	7.24±0.04	0.002
	MY 1821-2	8.25±0.05	7.6±0.04	7.45±0.05	7.2±0.05	0.004
	p value	0.072	0.074	0.248	0.340	
B. lactis	ABY3-1	7.54±0.04	7.45±0.1	6.98±0.06	6.16±0.08	0.003
	ABY3-2	7.35±0.05	7.25±0.05	6.8±0.05	6±0.055	0.002
	p value	0.007	0.035	0.019	0.047	
	MY 1821-1	7.38±0.04	7.48±0.04	7.65±0.04	6.9±0.06	0.009
	MY 1821-2	7.23±0.05	7.3±0.05	7.5±0.05	6.65±0.05	0.008
	p value	0.016	0.008	0.015	0.005	
L. casei	MY 1821-1	8.1±0.04	7.9±0.04	7.7±0.04	7.55±0.04	0.058
	MY 1821-2	8.05±0.05	7.8±0.05	7.6±0.05	7.55±0.04	0.019
	p value	0.840	0.054	0.054	1.00	

1. Mean value of 3 replicate.
2. *p* value less than 0.05 considered as significant.
3. 1: Uncontaminated samples, 2: contaminated samples
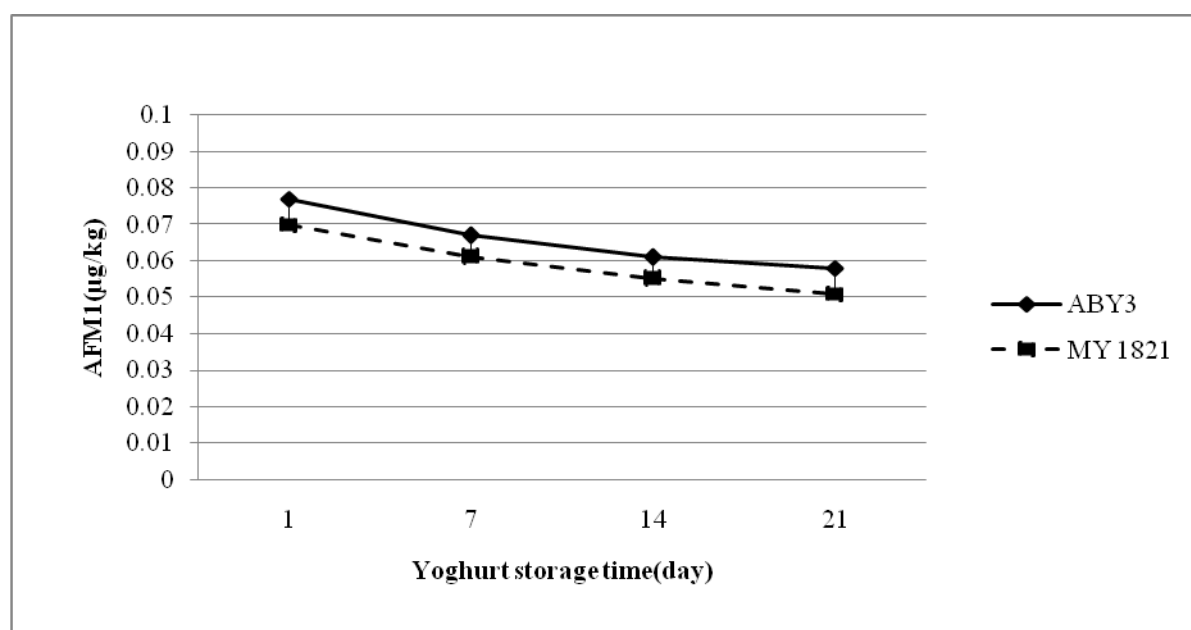
Fig. 2: Changes in AFM$_1$ concentration during storage of the probiotic yogurts (ABY3: culture containing *L. acidophilus*, *B .lactis*, *Str. thermophilus* and *L. bulgaricus*; MY 1821: culture containing *L. acidophilus* , *B. lactis* , *L. casei* , *Str. thermophilus* and *L. bulgaricus*.)

Behavior of AFM$_1$ during production and storage of the probiotic yogurts

Analysis of the reconstitute milk used for production of the yogurts showed that AFM$_1$ was not present before spiking. The AFM$_1$ levels in probiotic yogurt samples showed a significant decrease ($p<0.05$) from the level that was initially present in milk (Fig. 2).

The mean concentrations of AFM$_1$ in 1,7,14 and 21 days stored MY 1821 yogurt (0.070, 0.061, 0.055 and 0.051 µg/kg)

were lower than ABY3 (0.077, 0.067, 0.061 and 0.058 µg/kg), but pair ways comparison in each storage time showed that the differences were not significant ($p>0.05$). The trend of decrease in AFM_1 levels up to 21-days were statistically significant ($p<0.05$) in the ABY3 and MY 1821 yogurts.

Effect of AFM_1 on the viability and survival of probiotic bacteria during storage

The variation in viability of *L. acidophilus*, *B. lactis* and *L. casei* in the contaminated and uncontaminated yogurts during the 21-day refrigerated storage period are shown in Table 2.

The population of tested probiotic bacteria in the contaminated yogurts was lower than the control samples. The difference in viability of *L. acidophilus* in contaminated and control samples was not significant, except for 7 and 14-day-old ABY3 yogurt.

Among the tested strains, *B. lactis* showed minimum stability in presence of AFM_1 in all samples, the count of this bacteria was significantly lower than the control ($p<0.05$). As illustrated in Table 2, *L. casei* was the most stable species, so that, in comparison with other strains the population of this bacterium in the contaminated and uncontaminated sample remained nearly the same.

Discussion

The results of method validation showed that the method is capable to achieve accepted results for determination of AFM_1 in yogurt. This is the first assessment of the effect of probiotic yogurt on the AFM_1. Previous research in this field focused on the behavior of AFM_1 in nonprobiotic yogurt. Govaris et al. (2002) studied the stability of AFM_1 in yogurt artificially contaminated with concentrations of 0.050 and 0.100 µg/kg during storage for 4 weeks, at 4 °C, at two pH levels, viz. 4.0 and 4.6. They showed that at a pH of 4.6, the AFM_1 levels did not significantly change; however, in yogurt having a pH of 4.0, AFM_1 showed a significant decrease after the third and fourth weeks of storage at both concentration levels. Their results were to some extent in agreement with this work. Contrary to our findings, Iha et al. (2013) concluded that yogurt production and storage up to 28 days Had no significant effect on AFM_1 content. Bakirci (2001) found that the AFM_1 increased 13% higher than that of bulk-tank milk samples, but it was not statistically significant.

Decrease in AFM_1 levels in yogurt might be attributed to factors such as low pH, formation of organic acids or other fermentation by-products (Govaris et al., 2002). During fermentation the low pH alters the structure of milk proteins such as the caseins and lead to formation of yogurt coagulum; the changes in casein structure during yogurt production may affect the association of AFM_1 with this protein causing adsorption or occlusion of the toxin in the precipitate (Brackett and Marth, 1982). The acidity which develops in yogurt during fermentation may results in degradation of AFM_1 in yogurt (Rasic et al., 1991). Lactic acid bacteria that ferment milk to yogurt are capable to remove AFM1 from milk. El Khoury et al. (2011) reported that the yogurt bacteria, *L. bulgaricus*, *Str. thermophilus* and a combination of these two bacteria reduced AFM_1 content of milk as 58.5%, 37.7% and 46.7% respectively, after incubation in 37°C for 6 hours.

Several other fermentation by-products such as volatile fatty acids, amino acids, peptides or aldehydes could also account for degradation of AFM_1 in yogurt (Govaris et al., 2002). Presence of the probiotic bacteria could be an important factor in reducing the AFM_1 content of milk and dairy products. Preliminary investigations have shown that the probiotic bacteria can remove AFM_1 from milk *in vitro* and *in vivo* models. Elgerbi et al. (2006) assessed the ability of strains of *Lactobacillus* spp., *Lactococcus* spp. and *Bifidobacterium* spp. to bind the AFM_1 in solution. They found that the percentage of AFM_1 bound by these strains ranged from 4.5-73.1% after 96 h. Bovo et al. (2012) evaluated the ability of some probiotic strains to remove AFM_1 in skimmed milk and reported that the tested strains bound AFM_1 within a range from 13.51 to 37.75 % for 15 min at 37 °C. Serrano-Niño et al. (2013) assessed the ability of some spices of probitic bacteria and reported that the bioaccessability of AFM_1 reducded in range of 22.72 to 45.17% in presence of the tested strains. *In vitro* binding experiments demonstrate that viable probiotic bacteria can bind AFM_1 in reconstituted milk with ranging from 7.85 to 25.94% (Kabak and Var, 2008). Although the mechanism of action of these microorganisms on aflatoxin has not been clarified yet, it is thought that the primary cellular components involved are peptidoglycan, as well as cell wall polysaccharides and proteins. Substantial reduction in the AFM_1 level observed in this study may be due to use of high population of lactic acid and probiotic bacteria and long contact time between the bacteria and AFM_1, along with the effect of yogurt by-products.

Many factors such as production of hydrogen peroxide, reduction in pH, presence of lactic acid and the antagonistic effect between the probiotic and yogurt starter culture can decrease the viability of probiotic bacteria (Lourens-Hattingh and Viljoen, 2001); however the effect of AFM_1 on the survival of probiotics has not been studied yet. Very limited studies have shown that AFM_1 have some negative effect on yogurt starter cultures such as fermentability of starter culture (El-Deeb, 1989; Rasic et al., 1991; Sutic and Banina, 1979) and longer chains of bacteria in yogurt and yogurt-cheese (Coallier-Ascah and Idziak, 1985; Hassanin, 1994). Sutic and Banina (1979) have shown that morphological changes occurred for both streptococci and lactobacilli in yogurt samples containing higher concentrations of AFM_1. Yogurt having high concentration of AFM_1 showed

prolonged fermentation time compared to the control ones, due to difference in the growth rate of *S. thermophilus* (Coallier-Ascah and Idziak, 1985; Govaris et al., 2002). In addition, it has been reported that some of the homofermentative lactic acid bacteria were converted to hetrofermentative and produced gas (Sutic and Banina, 1990). Our result showed that AFM_1 had no remarkable negative effect on viability of the probiotic bacteria, because the population of the tested bacteria remained above the legislation for minimum viability of probiotic bacteria at the end of the experiments and the products were still probiotic.

Conclusion

The probiotic yogurt can reduce the AFM_1 content of initial milk during production and storage. Our result showed that the high population and multi-strain probiotic cultures results in a lower level of AFM_1 at the end of yogurt storage. Initial AFM_1 contamination of milk at level of 0.01μg/l and lower cannot negatively affect the survival of probiotic spices of yogurt culture. Therefore, it is recommended for dairy industry and food safety agencies, especially in regions with high level contamination of milk by AFM_1, to have screening programs for initial milk, and use high contaminated milk in production of yogurt and yogurt-like probiotic products. It should also be highlighted that the probiotic yogurt is safer for consumer than the nonprobiotic ones. As investigations in this issue are limited, more studies are needed to investigate the effectiveness of other mixed probiotic cultures with different composition, to reduce the AFM_1 content of milk.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgement

References

Bakirci I. (2001). A study on the occurrence of aflatoxin M_1 in milk and milk products produced in Van province of Turkey. *Food Control*. 12: 47-51.

Bovo F., Corassin C.H., Rosim R.E., Oliveira C.A.F. (2012). Efficiency of lactic acid bacteria strains for decontamination of aflatoxin M_1 in phosphate buffer saline solution and in skim milk. *Food and Bioprocess Technology*. 5: 1-5.

Brackett R.E., Marth E.H. (1982). Association of aflatoxin M_1 with casein. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*. 174: 439-441.

Coallier-Ascah J., Idziak E.S. (1985). Interaction between *Streptococcus lactis* and *Aspergillus flavus* on production of aflatoxin. *Applied and Environmental Microbiology*. 49: 163-167.

Corassin C.H., Bovo F, Rosim R.E., Oliveira C.A.F. (2013). Efficiency of *Saccharomyces cerevisiae* and lactic acid bacteria strains to bind aflatoxin M_1 in UHT skim milk. *Food Control*. 31: 80-83.

Creppy E.E. (2002). Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicology Letters*. 127: 19-28.

El-Deeb S.A. (1989). Interaction of afatoxin M_1 on the physiological and morphological properties of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Alexandria Journal of Agricultural Research*. 34: 61-72.

Elgerbi A.M., Aidoo K.E., Candlish A.A.G., Williams A.G. (2006). Effects of lactic acid bacteria and bifidobacteria on levels of aflatoxin M_1 in milk and phosphate buffer. *Milchwissenschaft*. 61: 197-199.

El Khoury A., Atoui A.,Yaghi J. (2011). Analysis of aflatoxin M_1 in milk and yoghurt and AFM_1 reduction by lactic acid bacteria used in Lebanese industry. *Food Control*. 22: 1695-1699.

European Commission. (2006a). Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union*. L364: 5-24.

European Commission. (2006b). Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. *Official Journal of the European Union*. L70: 12-34.

Fallah A.A. (2010a). Aflatoxin M_1 contamination in dairy products marketed in Iran during winter and summer. *Food Control*. 21: 1478-1481.

Fallah A.A. (2010b). Assessment of aflatoxin M_1 contamination in pasteurized and UHT milk marketed in central part of Iran. *Food and Chemical Toxicology*. 48: 988-991.

Fallah A.A., Rahnama M., Jafari T., Saei-Dehkordi S.S. (2011). Seasonal variation of aflatoxin M_1 contamination in industrial and traditional Iranian dairy products. *Food Control*. 22: 1653-1656.

Food and Agriculture Organization (FAO). (2001). Aflatoxin M1. In Safety evaluations of specific mycotoxins, prepared by the fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives, Geneva.

Ghazani M.H. (2009). Aflatoxin M_1 contamination in pasteurized milk in Tabriz (northwest of Iran). *Food and Chemical Toxicology*. 47: 1624-1625.

Govaris A., Roussi V., Koidis P.A., Botsoglou N.A. (2002). Distribution and stability of aflatoxin M_1 during production and storage of yoghurt. *Food Additives and Contaminants*. 19: 1043-1050.

Hassanin N.I. (1994). Stability of aflatoxin M_1 during manufacture and storage of yoghurt, yoghurt-cheese and acidified milk. *Journal of the Science of Food and Agriculture*. 65: 31-34.

Heshmati A, Milani J.M. (2010). Contamination of UHT milk by aflatoxin M_1 in Iran. *Food Control*. 21: 19-22.

Iha M.H., Barbosa C.B., Okada I.A., Trucksess M.W. (2013). Aflatoxin M_1 in milk and distribution and stability of aflatoxin M_1 during production and storage of yoghurt and cheese. *Food Control*. 29: 1-6.

International Agency for Research on Cancer (IARC). (2002). Monograph on the evaluation of carcinogenic risk to humans. vol. 82. World Health Organization, IARC, Lyon, France. p. 171.

Kabak B., Dabson A.D., Var I. (2006). Strategies to Prevent Mycotoxin Contamination of Food and Animal Feed: A Review. *Critical reviews in food science and nutrition*. 46: 593-619.

Kabak B., Var I. (2008). Factors affecting the removal of aflatoxin M_1 from food model by *Lactobacillus* and *Bifidobacterium* strains. *Journal of Environment Science and Health, Part B Pesticides*. 43: 617-624.

Kabak B., Ozbey F. (2012). Aflatoxin M_1 in UHT milk consumed in Turkey and first assessment of its bioaccessibility using an in vitro digestion model. *Food Control*. 28: 338-344.

Kurmann J.A., Rasic J.L. (1991). The health potential of products containing *bifidobacteria*. In R.K. Robinson (Ed.), Therapeutic properties of fermented milks (pp. 117-157). London, UK: Elsevier Applied Science Publishers.

Lourens-Hattingh A., Viljoen B.C. (2001). Yoghurt as probiotic carrier food. *International Dairy Journal*. 11: 1-17.

Mortazavian A.M., Ehsani M.R, Mousavi S.M., Rezaei K., Sohrabvandi S., Reinheimer J.A. (2007). Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt. *International Journal of Dairy Technology*. 60: 123-127.

Mortazavian A.M., Ehsani M.R, Sohrabvandi S., Reinheimer J.A. (2007). MRS-bile agar: its suitability for the enumeration of mixed probiotic cultures in cultured dairy products. *Milchwissenschaft*. 62: 270-272.

Murphy P.A., Hendrich S., Landgren C., Bryant C.M. (2006). Food mycotoxins: an update. *Journal of Food Science*. 71: 51- 65.

Neal G.E., Eaton D.L., Judah D.J., Verma A. (1998). Metabolism and toxicity of aflatoxins M_1 and B_1 in human-derived in vitro system. *Toxicology and Applied Pharmacology*. 151: 152-158.

Pitt J.I. (2000). Toxigenic fungi: which are important. *Medical Mycology*. 38: 17-22.

Prandini A., Tansini G., Sigolo S., Filippi L., Laporta M., Piva G. (2009). On the occurrence of aflatoxin M_1 in milk and dairy products. *Food and Chemical Toxicology*. 47: 984-991.

Rašić J.L., Škrinjar M., Markov S. (1991). Decrease of aflatoxin B_1 in yoghurt and acidified milks. *Mycopathologia*. 113: 117-119.

Serrano-Niño J.C., Cavazos-Garduño A., Hernandez-Mendoza A., Applegate B., Ferruzzi M.G., San Martin-González M.F., García H.S. (2013). Assessment of probiotic strains ability to reduce the bioaccessibility of aflatoxin M_1 in artificially contaminated milk using an in vitro digestive model. *Food Control*. 31: 202-207.

Shah N.P. (2000). Effects of milk-derived bioactives: An overview. *The British Journal of Nutrition*. 84: S3-S10.

Shah N.P. (2001). Functional food from probiotics and prebiotics. *Food Technology*. 55: 46-53.

Shah N.P. (2007). Functional cultures and health benefits. *International Dairy Journal*. 17: 1262-1277.

Sutic M., Banina A. (1979). The effect of aflatoxin B, on the morphological properties of lactic acid bacteria. *Mikrobiologija*. 16: 113-120.

Sutic M., Banina A. (1990). Influence of aflatoxin B_1 on gas production by lactic acid bacteria. *Journal of Environmental Pathology, Toxicology and Oncol*ogy. 10: 149-153.