Original Article

Reduction of Aflatoxin M1 in Milk Using Kefir Starter

Siavash Kamyar [1], Mohammadhosein Movassaghghazani[2]*

Received: 28.03.2017 *Accepted: 06.05.2017*

ABSTRACT

Background: Mycotoxins naturally occur in foods. Aflatoxins can cause serious health problems in consumers. Nowadays, biological detoxification method is considered to decrease the aflatoxins level in foods. The aim of this study was to evaluate the effect of kefir starter microorganisms to decrease the aflatoxin M1 (AFM1) levels in milk.

Methods: The study was carried out at Shabestar branch, Islamic Azad University in 2016. AFM1 at three levels 150, 200 and 250 ng/L was added to milk samples. Then a pool of lactic acid bacteria (LAB), yeasts and full kefir starter culture was added to milk samples. After cool storage of samples in 4 °C for 7 d, all samples were collected and the level of AFM1 determined by HPLC method. All samples were prepared in triplicate.

Results: The highest reduction percentage of AFM1 was observed in yeast (65.33%-68.89%) and LAB pool (65%). Samples with full kefir starter showed the reduction percent range of 11.67-34.66% that was lower in compare with other treatment groups.

Conclusion: These findings support the ability of LAB and yeasts to bind to aflatoxins in foods. Kefir drink in countries with high contamination by AFM1 in milk can be a safe dairy product choice for consumers.

Keywords: Aflatoxin M1, Detoxification, Kefir, Lactic Acid Bacteria, Yeast.

IJT 2017 (6): 27-31

INTRODUCTION

Aflatoxins are a class of toxic metabolites produced by *Aspergillus* species on foods and feed. Aflatoxins are responsible for damage to the crops. They have been associated with various diseases in livestock, domestic animals, and humans. Optimal thermal condition for *Aspergillus* growth and toxin production are 36 °C to 38 °C and 27 °C to 28 °C, respectively [1]. Aflatoxins are stable compounds and that are not destroyed during food processing [2, 3]. The occurrence of aflatoxins is differed by geographic location, agricultural and agronomic practices, and amount of fungal invasion during preharvest and storage. Aflatoxins are carcinogen in susceptible laboratory animals, and according to the International Agency for Research on Cancer (IARC) aflatoxins belong to group 1. It causes liver cancer in parts of the world where it is endemic [4].

There are four major aflatoxins: B1, B2, G1, G2 and two additional metabolic products, M1 and M2. Among six types of aflatoxins, B1 is the most carcinogens than others in human and animals. Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively. Whereas, aflatoxin M1(AFM1) is 4-hydroxy aflatoxin B1(AFB1) and aflatoxin M2(AFM2) is 4-dihydroxy aflatoxin B2(AFB2) [5].

Reports from Iran and some countries show that the AFM1 level in milk, dairy products, and infant formula is more than the maximum limit (50 ng/L) and it continues to be of great concern in milk consumers [2,3,6,7].

Kefir is a fermented milk product. The origin of kefir is from Caucasus region. Kefir grains are hard, small, yellowish-white granules. Three types of fermentation included lactic, alcoholic and acetic were performed by kefir starter microorganisms. Lactic acid is the major compound in kefir [8, 9].

Control of aflatoxins levels in foods is carried out by the prevention of mold contamination and detoxification of contaminated products. Physical methods of separation, thermal inactivation,

1. Department of Food Engineering, Shabestar Branch, Islamic Azad University, Shabestar, Iran.
2. Department of Food Hygiene and Food Quality Control, Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran.
*Corresponding Author: E-mail: movassagh2@yahoo.com

irradiation, microbial inactivation, and fermentation have been suggested by researchers. Some strains of lactic acid bacteria have the ability to bind aflatoxins in foods [10]. The mechanism of action is not clear. An adhesion to bacterial is the effect of lactic acid bacteria (LAB) on the aflatoxin reduction in fermented products. Some researchers have studied the ability of bacteria to decrease aflatoxins in milk and dairy products [10-12].

The aim of this research was to evaluate the kefir starter effects on the level of AFM1 in cow milk during kefir drink production and storage of kefir drink at 4 °C.

MATERIALS AND METHODS

Chemicals

The study was carried out at Shabestar branch, Islamic Azad University in 2016. Aflatoxin M1 was prepared from Sigma (Germany). AFM1 levels were 150, 200 and 250 ng/L. Each level was repeated for three times.

Kefir Starter Microorganisms

Kefir starter was prepared from CHR Hansen-Denmark. Three packages included: 1) F-DVS ABT-2 containing *Lactobacillus acidophilu*s, *Bifidobacterium* BB-12, and *Streptococcus thermophiles* (thermophilic lactic culture); 2) FD-DVS CHN-22 containing *Lactococcus lactis subsp. Cremoris, Leuconostoc*, *Lactococcus lactis subsp. Lactis biovar diacetylactis, Lactococcus lactis subsp. Lactis);* 3) LAF containing *Debaryomyces hansenii, Kluyveromyces marxianus subsp. Marxianus.*

For packages 1 and 2 of starter's packages, the incubation temperature was 22 °C for 6 h. Then the yeasts package was added and incubated at room temperature for production of gas in kefir drink.

Kefir Drink Preparation

Kefir drink production was according to the CHR Hansen procedure. For study, each package and full kefir starter were compared.

Kefir starter was added to cow milk (Nestle-Iran) that was free of AFM1. One hundred grams of milk powder were added to 1000 ml of deionized water, and milk was heated at 120 °C for 15 min and 0.01 gr of each starter's packages were added to 1000 mL of milk.

AFM1 was added at three levels to milk and kefir starter microorganisms were added to three types (Treatment) included Lactobacilli + Lactococci (T1), yeasts (T2) and full kefir starter (T3). For each level of AFM1 control sample(C) was prepared. Kefir starter microorganisms were not added to control samples. The milk was allowed to ferment and the kefir fermentation was considered complete with pH of 4.6. Samples were collected after 7 d maintenance at 4 °C. The amount of AFM1 was detected by HPLC method. All samples were prepared in triplicate. Samples' pH determination was carried out according to ISIRI. No.2852 with pH meter (HORIBA, Japan) at 1st and 7th d.

HPLC Method

The HPLC method was validated according to ISIRI. No. 7133. Three steps included extraction, purification, and quantitative determination was used to measure the AFM1 level in samples by HPLC. Samples fatty layer was removed by centrifugation at 2500 gr for 10 min. Waters HPLC system (Waters co., Milford, MA. USA) with Waters 2475 detector were used for AFM1 detection. The analytical column was C 18 column (ODS-2) [13,14].

Statistical Analysis

For statistical analysis SPSS software ver. 16 (Inc., Chicago, IL, USA) was used and ANOVA and independent *t*-test were performed. Results with $P<0.05$ were considered statistically significant.

RESULTS

Comparing three treatment groups of microorganisms with statistical analysis showed that there were significant differences between the amounts of AFM1 in all strains. The results in Table 1 show the effect of pH on AFM1 reduction in samples treated by microorganisms. The overall rates for AFM1 in kefir drink have been shown in Table1.

The reduction rate of AFM1 kefir treatments has been shown in Figure 1. The highest reduction percentage was seen in LAB pool and yeast strains with the different amount of initial AFM1. The initial amount of AFM1 at the common level of the strains' amount in milk is not effective on the degradation percentage of AFM1 in samples.

The full stains' samples compared to each strain group showed the lower reduction percentage of AFM1 (Figure 1).

Table 1. Average mean values of AFM1 in kefir treatments, T1: Lactobacilli + Lactococci, T2: yeasts, T3: full kefir starter.

Treatment	Initial AFM1 in milk(ng/L)	pH	Average Mean values of AFM1 in kefir treatments(ng/L) mean±SEM	pH	*P* value
Control	150	5.84	150	5.94	-
	200	6.07	200	6.05	-
	250	6.05	250	4.93	-
T1	150	4	63.33±3.33**	3.94	0.001
	200	4.04	70±5.77**	4.04	0.001
	250	4.07	113.33±13.33^	3.95	0.009
T2	150	4.17	46.66±8.81*	4.18	0.007
	200	4.21	110±15.27*	4.52	0.028
	250	4.38	86.66±12.01*	4.68	0.005
T3	150	4.02	120±11.54	3.98	0.060
	200	4.10	176.66±3.33*	4.01	0.020
	250	4.02	163.33±6.66*	3.97	0.006

**P<0.001, *P<0.05

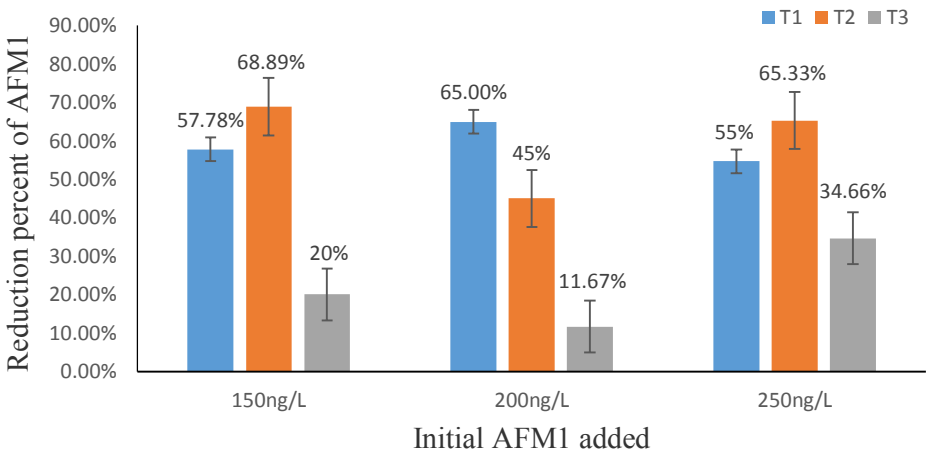

Figure 1. Reduction rates of AFM1 in kefir treatments.

T1: Lactobacilli + Lactococci; T2: yeasts; T3: full kefir starter

DISCUSSION

The percentages of AFM1 reduction in kefir drink treatments in the present study are in agreement with the results of others [10,15]. The maximum reduction rate of AFM1 was at 20% kefir sample and between bacterial samples; *L. casei* had the maximum reduction. The added yeast to LAB was increased the reduction rate of AFM1 in samples [16]. At present study, the maximum reduction was in samples with yeast. Samples contained LAB (lactobacilli + lactococci) showed high reduction rate of AFM1 at the toxin group involved of 200 ng/L.

The ability of *S. cerevisiae* strain was studied and a pool of lactic acid bacteria strains in reduction of AFM1 in UHT skimmed milk. Using *S. cerevisiae* with LAB pool increased the level of AFM1 during 60 min by 100% [15]. In our study, *Debaryomyces hansenii* and *Kluyveromyces marxianus subsp. Marxianus.* showed the highest reduction of AFM1 level. Yeast strains have been investigated as an aflatoxin binder[17]. Studies mainly focus on *S. cerevisiae*. The reduction rate depends on strain specific and varies among different strains[17].

Table 2 gives a range of reduction percentage in aflatoxins level from some reports about the effects of fermentation on aflatoxins in milk.

The reduction of AFM1 in different kefir drink might be due to binding AFM1 to bacterial cell wall components and low pH in kefir [18]. Low pH can cause to degradation of AFM1 in kefir drink. On the other hand, the oxidation of glucose produces hydrogen peroxide in kefir drink and the reactive oxygen will react with AFM1 molecule[10].

The effect of pH on in vitro reduction of aflatoxin B1 was studied by strains of LAB isolated from Moroccan sourdough bread and the highest removal of AFB1 was at pH range from 3 to 4.5 [19].

Yogurt fermented by 50% yogurt culture and 50% *L. planetarium* had the highest reduction at the rate of AFM1 at the end of a storage period. Similar results was obtained at present study [10].

Different strains of LAB had distinct reduction percentage of AFM1. There are some reports that the fermentation showed no effect at the level of AFM1 in foods [18]. Our findings support the ability of LAB and yeasts to bind to mycotoxins in foods.

Table 2. Examples of reported effects of microorganism's fermentation on aflatoxins.

Toxin type	Raw materials	Product	Type of fermentation	Percent of reduction	Reference
Aflatoxin M1	milk	Yogurt	lactic	69.8-87.8%	[10]
Aflatoxin M1	milk	Yogurt	lactic	26.2-34%	[20]
Aflatoxin M1	milk	UHT milk	lactic	11.7%	[15]
Aflatoxin M1	milk	UHT milk	Lactic+Yeast	100%	[15]
Aflatoxin M1	milk	UHT milk	Yeast	92.7%	[15]
Aflatoxin M1	milk	Kefir	Lactic+Yeast	91.91%	[16]
Aflatoxin B1	PBS	PBS	Lactic	5.6-25.7%	[21]
Aflatoxin B1	PBS	PBS	Lactic	50%	[21]

CONCLUSION

LAB and yeasts can be used in dairy product to reduce the AFM1 in milk. Furthermore, the kefir drink use can improve the health conditions of dairy consumers. The mechanism of removal effect of yeast is not clear and additional studies are needed. Overall, microorganisms in the kefir starter have the ability to reduce the AFM1 amount to safe level in countries that the milk and dairy products have shown high contamination by AFM1 like as Iran. Although the kefir starter reduces toxicity, it is not able to complete detoxification. Additional studies are recommended to investigate the other strains of bacteria and yeasts' mechanism on mycotoxins reduction in foods.

ACKNOWLEDGMENTS

This paper is the result of MSc thesis in Food Science and Technology. The authors thank Dr. Shojaee (Faroogh Laboratory) for his help in HPLC analysis. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

The authors declare that there is no conflict of interest.

REFERENCES

1. Shepherd GS. Aflatoxin and Food Safety: Recent African Perspectives. In: Abbas HK, editor. Aflatoxin and Food Safety. USA: CRC Press; 2005. p.13-27.
2. Rohani FG, Aminaee MM, Kianfar M. Survey of aflatoxin M1 in cow's milk for human consumption in Kerman Province of Iran. Food Addit Contam Part B 2011;4(3):191-4.
3. Ghazani MHM. Aflatoxin M1 contamination in pasteurized milk in Tabriz (northwest of Iran). Food Chem Toxicol 2009;47(7):1624-5.
4. Hashemi M. A survey of aflatoxin M1 in cow milk in Southern Iran. J Food Drug Anal 2016;24(4):888-93.
5. Samanta I. Veterinary Mycology. India:Springer India; 2015.
6. Torović L. Aflatoxin M1 in processed milk and infant formulae and corresponding exposure of adult population in Serbia in 2013-2014. Food Addit Contam Part 2015;8(4):235-44.
7. Mokoena M, Chelule P, Gqaleni N. The toxicity and decreased concentration of aflatoxin B1 in natural lactic acid fermented maize meal. J Appl Microbiol 2006;100(4):773-7.
8. Chandan RC, Shahani KM. Yogurt. In: Hui YH, editor. Dairy Science and Technology Handbook. Wiley, New York, USA; 1992. p. 359-63.
9. Puniya AK. Fermented milk and dairy products. CRC Press; 2015. P. 13-23.
10. Elsanhoty RM, Salam SA, Ramadan MF, Badr FH. Detoxification of aflatoxin M1 in yoghurt using probiotics and lactic acid bacteria. Food control 2014;43:129-34.
11. Shahin A. Removal of aflatoxin B1 from contaminated liquid media by dairy lactic acid bacteria. Int J Agric Biol 2007;9:71-5.
12. Galvano F, Piva A, Ritieni A, Galvano G. Dietary strategies to counteract the effects of mycotoxins: a review. J Food Prot 2001;64(1):120-31.

13. Fernandes A, Corrêa B, Rosim R, Kobashigawa E, Oliveira C. Distribution and stability of aflatoxin M 1 during processing and storage of Minas Frescal cheese. Food control 2012;24(1):104-8.
14. Dragacci S, Grosso F, Gilbert J. Immunoaffinity column cleanup with liquid chromatography for determination of aflatoxin M1 in liquid milk: collaborative study. J AOAC Int 2001;84(2):437-43.
15. Corassin C, Bovo F, Rosim R, Oliveira C. Efficiency of *Saccharomyces cerevisiae* and lactic acid bacteria strains to bind aflatoxin M 1 in UHT skim milk. Food control 2013;31(1):80-3.
16. Isakhani S, Marhamatizadeh MH, Tajabadi-Ebrahimi M. The assessment of reducing aflatoxin M1 in kefir by *Saccharomyces kefir* and *Lactobacillus casei* TD4 by ELISA method. Trends Life Sci 2014;3(4):268–74.
17. Guan S, Zhou T, Yin Y, Xie M, Ruan Z, Young J. Microbial strategies to control aflatoxins in food and feed. World Mycotoxin J 2011;4(4):413-24.
18. Shetty PH, Jespersen L. *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. Trends Food Sci Technol 2006;17(2):48-55.
19. Zinedine A, Faid M, Benlemlih M. In vitro reduction of aflatoxin B1 by strains of lactic acid bacteria isolated from Moroccan sourdough bread. Int J Agric Biol 2005;7(1):67-70.
20. Maryamma K, Rajan A, Gangadharan B, Ismail P, Valsala K, Manomohan C. Reduction of aflatoxin in milk by fermentation into curd. J Vet Anim Sci 1990;21(2):102-7.
21. El-Nezami H, Kankaanpaa P, Salminen S, Ahokas J. Ability of dairy strains of lactic acid bacteria to bind a common food carcinogen, aflatoxin B 1. Food Chem Toxicol 1998;36(4):321-6.