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Aflatoxin M_1 contaminations in mouldy cheese

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Abstract

In this study, a total of 100 mouldy cheese samples were collected from randomly selected markets from 5 different cities of Turkey during October and November. Samples were analyzed for aflatoxin M_1 by using enzyme linked immunosorbent assay (ELISA) technique. Aflatoxin M_1 was detected in 52 samples and their levels ranged from 10.6 to 702 ng/kg. The widespread presences of aflatoxin M_1 in mouldy cheese may be considered to be potential hazards for human health.

Key words: mouldy cheese, aflatoxin M_1, ELISA, mycotoxin

Introduction

Aflatoxins are toxic secondary metabolites produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Mohajeri et al., 2013). The common types of aflatoxins are B_1, B_2, G_1, G_2, M_1 and M_2. Aflatoxin M_1 and M_2 (AFM_1, AFM_2) are hydroxylated metabolites of aflatoxin B_1 and B_2 (AFB_1, AFB_2), respectively. When the animals consume feeds contaminated with AFB_1 or AFB_2, their milks contain AFM_1 or AFM_2 (Anfossi et al., 2012; Bilandžić et al. 2010). If contaminated milk is used in cheese production, toxins can be carried over into cheese. Pasteurization or sterilization cannot destroy aflatoxins because these compounds are thermostable (Picinin et al., 2013). Aflatoxins can cause dangerous illnesses, they are carcinogenic, mutagenic and teratogenic compounds (Bilandžić et al., 2010). The most toxic aflatoxin type is AFB_1 which is classified to be group 1 carcinogenic compounds for humans by International Agency for Research on Cancer (IARC) of World Health Organization (WHO) (IARC 2002). AFM_1 is less toxigenic than AFB_1. However, AFM_1 is also classified as group 1 carcinogenic compounds for humans by IARC because of its hepatotoxic and carcinogenic effects (IARC, 2002).

Human can be exposed to AFM_1 by consuming dairy products. Aflatoxin concentration in cheese is about four times higher than in milk. The increase in AFM_1 concentration has been explained by the affinity of AFM_1 for casein (Sengun et al., 2008).

Cheese has the highest consumption rate among dairy products. A large number of traditional cheese varieties are produced in Turkey. Mouldy cheese is one of the traditionally produced cheeses. Cheeses are ripened with naturally occurring moulds. No mold culture is added during ripening. These moulds grow inside of the cheese naturally. Characteristic properties of mouldy cheeses are shown in Table 1.

The aim of this study was to determine concentration of AFM1 in mouldy cheese (Isparta mouldy comlek cheese, Konya mouldy cheese, Erzurum mouldy civil cheese, Mersin mouldy tulum cheese, Kayseri mouldy cheese) that are traditionally produced in different regions of Turkey with different production method. Many studies have been conducted to determine AFM_1 contamination in cheese, but there is lack of studies about mouldy cheese in the literature. In this respect, this study is notable.

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Materials and methods

Material

Samples

A total 100 samples of mouldy cheese were obtained from five different cities with the highest mouldy cheese production capacity (Konya, Mersin, Kayseri, Isparta and Erzurum) of Turkey. The samples of 1000 g were taken from randomly selected markets. The samples were transported laboratory within an insulated container.

Reagents

All reagents were analytically pure and obtained from Merck KGaA (Darmstadt, Germany). ELISA test kits were purchased from R-Biopharm AG (Darmstadt, Germany) and they are stored at 4 °C before use.

Method

AFM_1 contents of samples were determined by using ELISA method (RIDASCREEN Aflatoxin M1 30/15, Art. No:R 1111, Darmstadt, Germany). The calibration curve was virtually linear, ranging 5-80 ng/kg. In order to obtain the AFM_1 concentration in ng/kg in cheese, the concentration read from the calibration curve must be multiplied by dilution factor. According to the instructions for use of the RIDASCREEN kit, detection limit of cheese is 50 ng/kg. The recovery rate is 102 % with a mean coefficient of variation of 11 %.

Preparation of cheese samples

All samples were prepared according to the instructions of the RIDASCREEN test kit. Each sample was coarsely triturated and thoroughly mixed. Two g of cheese sample and 40 mL of dichloromethane were added to the vial and shaken for 15 min. The suspension was filtered and 10 mL extract evaporated at 60 °C under nitrogen. The oily residue was redissolved in 0.5 ml methanol, 0.5 mL PBS buffer and 1 mL heptane and centrifuged at 2700 g for 15 min. The upper heptane layer was completely removed. Aliquot of the lower methanolic-aqueous phase was poured off with using pasteur pipette. 100 μL of this aliquot was diluted with 400 μL buffer 1 and 100 μL dilution was used for per well (Anonymus, 2009).

ELISA test procedure

A hundred μL of the standard solutions (0, 5, 10, 20, 40 and 80 ng/kg) and samples were placed to microtiter wells. The plate was shaken and incubated at room temperature in the dark for 30 min. Then it was washed twice with washing buffer. One hundred μL of diluted enzyme conjugate was added to wells. The plate was shaken and incubated at room temperature in the dark for 15 min. Afterwards the plate was washed twice with washing buffer again. One hundred μL of substrate/chromogen was added to each well. The plate was shaken and incubated at room temperature in the dark for 15 min. Finally, 100 μL of the stop solution was added to wells and the plate was shaken. The plate was read within 15 minutes at 450 nm absorbance (Anonymus, 2009).

Table 1. Characteristics properties of mouldy cheese samples

Cheese type	Characteristics
Isparta mouldy comlek (earthenware pot) cheese	Curd is mixed with strained yoghurt and black cumin and dried under the sun. This mix is filled into earthenware pot, and ripened for 3-4 months in soil
Konya mouldy cheese	Curd is ripened in goat or sheep tulum (skin) for 3-4 months
Erzurum mouldy civil cheese	Curd is heated in the whey, after kneaded curd ripened in plastic can for 2 month
Mersin mouldy tulum cheese	Curd is crumbled and salt is added then ripened in tulum (skin) for 1 month
Kayseri mouldy cheese	Curd is mixed with tallow then ripened in earthenware jar for 3-4 months

Statistical analysis

Statistical analysis was performed by using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare AFM_1 levels of cheese. The levels were considered significantly different at $p<0.05$.

Results and discussion

In this study, a total of 100 mouldy cheese samples were analyzed by ELISA. It is determined that 52 % of all mouldy cheese samples contaminated by AFM_1 and concentrations ranging from 10.6 to 702 ng/kg. Number of aflatoxin positive samples is shown and levels of positive samples are shown in Table 2 and 3.

The average AFM_1 level in positive samples was determined as 211 ng/kg. The highest mean value for AFM_1 was found in Mersin mouldy cheese. Seven of the 17 positive Mersin mouldy cheese samples concentration levels were higher than 500 ng/kg.

All of the mouldy cheese samples from Mersin region were contaminated by aflatoxin M_1 and 85 % of cheese samples had AFM_1 contamination above Turkish Regulations (250 ng/kg). The highest AFM_1 contamination level was detected in mouldy cheese collected from Mersin region (702 ng/kg). That is the most hazardous mouldy cheese compared to others. Mersin is located at the coastal area and during the summer months the weather is hot and humidity is high in this region. These adverse effects can influence on the development of moulds in the stored feed. It should be considered that the mouldy feed for animals intended for breeding is the most important risk factor for AFM_1 in milk and dairy products.

On the other hand, AFM_1 was not detected in any of the Erzurum mouldy cheese samples. This region is the one in which AFM_1 could not be determined. The ratio of contaminated Konya mouldy cheese samples by AFM_1 is 25 %. It should be noted that concentrations of contaminated samples range from 32.7 to 58.5 ng/kg.

Table 2. Occurrence of AFM_1 in mouldy cheese samples

Cheese variety	Number of samples	Positive samples
Erzurum mouldy cheese	20	0
Konya mouldy cheese	20	5
Isparta mouldy cheese	20	17
Mersin mouldy cheese	20	20
Kayseri mouldy cheese	20	10
Total mouldy cheese	100	52

Table 3. AFM_1 levels of positive cheese samples

Cheese variety	Positive samples		
	Mean±SD (ng/kg)	Maximum (ng/kg)	Minimum (ng/kg)
Erzurum mouldy cheese	-	-	-
Konya mouldy cheese	41.4^{b}±10.9	58.5	32.7
Isparta mouldy cheese	132^{b}±86.9	298	17.9
Mersin mouldy cheese	405^{a}±170	702	119
Kayseri mouldy cheese	38.5^{b}±53.3	188	10.6
Average AFM_1content	211	702	10.6

-: Not detected
SD: Standard Deviation
^{a,b} Different letters show the significant difference ($p>0.05$)

AFM_1 was found in 17 mouldy cheese samples from Isparta. Concentrations of samples ranged between 17.9 and 298 ng/kg. Also, 50% of Kayseri mouldy cheeses were contaminated by AFM_1.

Statistical evaluation has showed that there were no significant differences between AFM_1 contents of cheese collected from Konya, Kayseri and Isparta regions. However, Mersin cheese were statistically different from other positive cheese samples ($p<0.05$).

Some results of AFM_1 contamination in cheese have been reported in different countries. Commission of European Communities and Turkish Food Codex (Anonymus, 2011) has set a limit of 50 ng/L for AFM_1 in raw milk, heat treated milk and milk for the manufacture of milk based products. But limit of AFM_1 in cheese changes depending on cheese varieties (Anonymus, 2010). Specific limits for AFM_1 in cheese have been introduced by some countries such as Switzerland, Austria, France, Iran (250 ng/kg), Netherlands (200 ng/kg), Italy (450 ng/kg) (Durakovic et al., 2009; Cavallarin et al., 2014; Fallah, 2010; Skrbic et al., 2015).

Seventy-two white cheese samples were analyzed for AFM_1 in Iran. The toxin was detected in 59 samples and 22 of them exceeded the Iranian national standard limit (Fallah, 2010). In Lebanon, AFM_1 was detected in 75 out of 111 cheese samples. Thirteen of the samples were found to exceed the limit of 250 ng/kg (Elkak et al., 2012). In Kuwait, 40 different cheese samples were analyzed for AFM_1 contamination. Eighty percent of these samples tested positive of AFM_1 and its levels ranging from 23.8 to 452 ng/kg (Dashti et al., 2009). In Serbia 54 samples of hard and white type of cheese were investigated for AFM_1 contamination and seven of them exceeded the limit of 250 ng/kg (Skrbic et al., 2015). In Brazil AFM_1 contamination were tested in 30 Parmesan cheese samples. AFM_1 was detected in 18 of the evaluated samples and 8 of them exceeded the limit of 250 ng/kg (Trombete et al., 2014).

In the previous studies different types of cheese were investigated for AFM_1 contamination in Turkey (Table 4). AFM_1 contamination levels of positive samples ranged between 12 and 800 ng/kg. Different levels of AFM_1 in cheeses could be due to different production procedures, cheese production conditions, level of contamination in milk and ripening conditions (Sarimehmetoglu et al., 2004).

Table 4. AFM_1 levels of cheese in previous studies

Cheese variety	Number of samples	Positive samples number	Range (ng/kg)	References
Tulum cheese	20	16	13-378	Ertas et al., 2011
White cheese	20	14	15.6-154.6	
Kashar cheese	20	8	12-369.5	
White brined Urfa cheese	127	36	70.61-770.97	Kav et al., 2011
White brined cheese	193	159	52-860	Ardic et al., 2009
Kashar cheese	132	109	50-690	Tekinsen and Eken, 2008
Cream cheese	49	44	NR	Aycicek et al., 2005
White cheese	94	86		
Kashar cheese	53	47		
White cheese	200	10	100-600	Yaroglu et al., 2005
Kashar cheese	200	12	120-800	
Cream cheese	200	8	100-700	
White cheese	100	82	NR	Sarımehmetoglu et al., 2004
Tulum cheese	100	81		
Kashar cheese	100	85		
Processed Cheese	100	79		

NR: Not Reported

Conclusions

Existence of AFM_1 in mouldy cheese is serious problem regarding human health. Some precaution should be taken to prevent hazardous effect of AFM_1. There is a relationship between the AFM_1 content in milk used in making cheese and the AFB_1 content in feed. Therefore, contamination of animal feed by AFB_1 should be prevented. In addition, hygiene conditions and control of whole process should be taken into account in the production of mouldy cheese.

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Sirevi s plijesnima i aflatoksin M_1

Sažetak

Za ovo istraživanje prikupljeno je 100 sireva s plemenitim plijesnima s nasumično odabranih tržnica iz 5 gradova u Turskoj u vremenskom periodu od listopada do studenog. U sirevima je istražena prisutnost aflatoksina M_1 upotrebom ELISA metode. Aflatoksin M_1 je utvrđen u 52 uzorka, u koncentracijama od 10,6 do 702 ng/kg. Prisutnost aflatoksina M_1 u sirevima s plemenitim plijesnima može biti potencijalna opasnost za zdravlje ljudi.

Ključne riječi: sir s plemenitim plijesnima, aflatoksin M_1, ELISA, mikotoksin

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