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Determination of Aflatoxin M1 Levels and Antibiotic Residues in the Traditional Turkish Desserts and Ice Creams Consumed in Burdur City Center

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Abstract: Aflatoxins display an insufficiently recognized risk to human health. Aflatoxins M1 and M2 are oxidative metabolites of aflatoxins B1 and B2 in milk. They can be found in a variety of food items and are not destroyed by normal industrial processing or cooking. Also, foods from animals may contain residues of antibacterial drugs. In this study, totally 47 traditional milky dessert and ice cream samples including sutlac, gullac, muhallebi and ice cream were investigated for aflatoxin M1 by ELISA and for antibacterial drugs by bacterial growth inhibition methods. Aflatoxin values of the 8 samples exceeded 50 ng kg^{-1} and among them only one sample exceeded 250 ng kg^{-1}. Thirty-one of the 47 milky dessert samples were positive. According to the results, antibiotic residues were detected in 32 out of 47 samples of milky desserts (68.1%). The milky desserts including aflatoxin M1 and/or antimicrobial drug residues are potential risk for public health. Prevention of the contamination of aflatoxin B1 and B2 in the animal food may prevent formation of aflatoxin M1 in animal products. Also, the farmers should be educated for the prevention of antibiotic residues in milk.

Key words: Aflatoxin M1, antimicrobial residue, milky desserts, burdur, ice cream, animal food

INTRODUCTION

Aflatoxins display an insufficiently recognized risk to human health. They are formed in stored nuts, cereals and rice under high humidity and temperature conditions. The two major *Aspergillus* species that produce aflatoxins are *Aspergillus flavus* which produces only B aflatoxins and *Aspergillus parasiticus* which produces both B and G aflatoxins. Aflatoxins M1 and M2 are oxidative metabolites of aflatoxins B1 and B2 thus appear in milk, urine and faeces. Aflatoxins are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic compounds. The main target organ for toxicity and carcinogenicity is the liver (Peraica *et al*., 1999). They can be found in a variety of food items and since they are heat-stable, they can not be destroyed by normal industrial processing or cooking (Creepy, 2002).

Aflatoxin M1 levels in the milk and the milk products of Turkey were invastigated in some studies (Tekinsen and Tekinsen, 2005; Yaroglu *et al*., 2005; Kart *et al*., 2008; Tekinsen and Ucar, 2008; Tekinsen and Eken, 2008; Yapar *et al*., 2008; Aygun *et al*., 2009). However, there is no study concerning aflatoxin levels of traditional milky desserts muhallebi, sutlac and gullac.

Meat and other dietary products from food animals and farmed fish may contain residues of many antibiotics and antibacterial agents. A notable proportion of the general population has allergic sensitivity to these substances due to the prior medical treatments. However, the allergy to antimicrobial residues in food is seldomly reported (Dayan, 1993).

Aflatoxin M1 levels were detected and antibiotic residues in traditional milky desserts (muhallebi, sutlac, gulac and ice creams that were obtained from Burdur city center which is an important city of the Turkey in terms of the milk production. Cow's milk is produced abundantly and collectively in Burdur. The cooperatives of milk producers function efficiently (Celik, 2002).

Gullacis a Ramadan dessert which consists of very thin large dough layers in the milk and rose water, served with pomegranate seeds and walnut. Muhallebi is a sweet milk pudding dessert and sulacis also a sweet milk pudding dessert with rice.

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MATERIALS AND METHODS

Samples: Burdur is a small city in Turkey. A total of 47 milky dessert samples that were obtained from all of the patisseries in the city center were evaluated in the experiments. These milky desserts were traditional made ice cream and traditional Turkish desserts muhallebi, sulacand gulac Muhallebi and sulacare similar milky desserts so that they are in the same groupas shown in Table 1. Dessert samples were bought with their original boxes and carried in a cold storage box. Analysis were achieved immediately.

Aflatoxin assays: Helica Biosystems Mycomonitor Aflatoxin M1 ELISA kit (Helica Biosystems Inc. Fullerton, CA. USA) was used for quantitative detection of aflatoxin M1 in the samples following manufacturer's instructions. The microwells were measured at 450 nm by an Elisa reader. The optical densities of the samples were compared with the OD's of the kit standarts and an interpretative result was determined.

Ice cream, muhallebi and sulacsamples were exposed to some pretreatments. One gram of these desserts were mixed with 5 mL of absolute methanol in a capped tube for 5 min. The tubes were clarified by centrifugation (5000 g for 5 min) and 0.5 mL of supernatant was transferred to a glass tube then methanol was evaporated. About 0.25 mLof the provided blank (aflatoxin free) skim milk was added to deposited semi solid viscous material on the inside of the tube and vortexed for a min. These milk extracts were used in the above mentioned assay. Gullac samples were not exposed any treatment before Elisa, the milk samples were taken directly. Because gullac consists of very thin large dough layers in the milk.

Antimicrobial residue control: Bio-X Diagnostics Bio-X Chrom-AB Tube Kit (Bio-X Diagnostics, Belgique) was used for qualitative detection of total antibiotics and sulphonamides in the samples according to manufacturer's instructions with some pretreatmental modifications. The kit was consist of tube strips filled with an agar medium that incorporates a fermentable sugar, glucose and a pH indicator, bromocresol purple and pre-seeded spores of *Bacillus stearothermophilus* var. *Calidolactis*. The milk samples from gullac were pipetted directly on the surface of the agar. The samples which were taken from the muhallebi, sulac and ice cream samples were exposed to some pretreatments. An extraction buffer (from Bio-X Diagnostics, total antibiotic detection kit) was added, vortexed and incubated over night. The clear supernatant was pipetted on the surface of the agar in the tubes of the kit. Tubes were incubated at 65°C in a water bath. The sample quickly diffused through the agar medium. If there were no antimicrobials in the pipetted samples or the antimicrobial concentration was lower than the detection limits of the test, the *Bacillus* spores were able to germinate, grow and metabolize the sugar resulting with an intense yellow color due to the production of acid from utilized glucose. Antibiotic detection limit of the assay are as follows: Penicilin 2-4 ng mL^{-1}, ampicillin 3-4 ng mL^{-1}, amoxicillin 3-4 ng mL^{-1}, oxacillin 10-20 ng mL^{-1}, cloxacillin 15-40 ng mL^{-1}, dicloxacillin 10-20 ng mL^{-1}, nafcillin 5-15 ng mL^{-1}, streptomycin 400-800 ng mL^{-1}, dihydrostreptomycin 400-800 ng mL^{-1}, kanamycin 150-300 ng mL^{-1}, neomycin 50-150 ng mL^{-1}, erytromicin 100-200 ng mL^{-1}, tylosin 40-50 ng mL^{-1}, gentamycin 25-100 ng mL^{-1}, spectinomycin 200-400 ng mL^{-1}, marbofloxacin 800-1600 ng mL^{-1}, spiramicin 600-900 ng mL^{-1}, rifampicin 100-150 ng mL^{-1}, tetracycline 100-200 ng mL^{-1}, oxytetracycline 100-200 ng mL^{-1}, chlortetracycline 100-200 ng mL^{-1}, chloramphenicol 2500-3500 ng mL^{-1}, bacitracin 1500-3000 ng mL^{-1}, sulphametazine 100-200 ng mL^{-1}, sulfadiazine 25-100 ng mL^{-1}, sulfadimetoxina 25-100 ng mL^{-1}, sulfamerazine 25-100 ng mL^{-1}, sulfamethoxazole 25-100 ng mL^{-1}, sulfaquinoxalina 25-100 ng mL^{1}, trimethoprim 100-200 ng mL^{-1}, danofloxacin 800-1600 ng mL^{-1}.

RESULTS AND DISCUSSION

Aflatoxin M1 levels of the milky desserts were shown in the Table 1. Aflatoxin values of 8 samples (3 muhallebi and sutlac, 5 gullac) exceeded 50 ng kg^{-1}. Only one sample exceeded 250 ng kg^{-1} (gullac), the limit determined for dairy products by Turkish food codex (TFCB, 2002).

Table 1: Aflatoxin M1 levels of the 3 groups of milky desserts and antimicrobial positive samples

Samples	Number of samples (n)	Aflatoxin M1 positive samples (n)	Aflatoxin Levels Mean±SE (ng kg^{-1})	Min.	Max.	Antimicrobial positive samples (n)
Ice cream	16	6	6.06[a]±2.18	UDL	26.18	6
Muhallebi and Sutlac	21	15	32.3[b]±11.80	UDL	206.1	16
Gullac	10	10	83.5±33.20	12.4	352.9	10
Total	47	31	34.25±9.51	UDL	352.9	32

Value is statistically different from Muhallebi-Sutlac and Gullac groups ($p<0.05$), [b]Value is statistically different from Ice cream and Gullac groups ($p \leq 0.05$)
SE: Standard Error ; Min: Minimum; Max: Maximum; UDL: Under the Detection Limit

About 16 of the 47 desserts were negative. Lower values in the ice creams may be considered as normal since the milk content in ice cream is lower than that of muhallebi, sutlac and gullac. Gullac has the highest milk content as compared to other groups.

Kart *et al*. (2008) reported that 62.5% of the milk powder samples consumed in Turkey was positive for aflatoxin M1. This result is in agreement with the study as aflatoxin M1 was detected in 65.96% of the samples in the research. In related studies, Akdemir and Altintas (2004) reported that 70.83% of the milk samples coming from 12 different villages to 2 different milk factory was contained aflatoxin M1. In another study, 59.3% of the milk samples collected from Ankara were positive for aflatoxin M1 (Gurbay *et al*., 2006). In a study published in 1988, milk and cheese samples from Germany, France and Netherlands were evaluated in Italy. The percentages of aflatoxin M1 positive samples were 13.8 and 12.5% in German and French milks, respectively (Piva *et al*., 1988). These contamination levels are lower than the milk sample results of the present study.

We determined antibiotic residues in 32 samples of milky desserts (68.1%) whereas 15 were negative. There are few studies in Turkey considering antibiotic residues in milk. One of them reported that 66.8% of the milk samples were contaminated with various amounts of chloramphenicol, streptomycin and tetracycline (Unusan, 2009). Antibiotic contamination in foods may be important for some people have allergic sensitivity to these substances due to the prior medical treatments.

CONCLUSION

In this study, the milky desserts including aflatoxin M1 and/or antimicrobial drug residues are potential risk for public health. Prevention of the contamination of aflatoxin B1 and B2 in the animal food may prevent formation of aflatoxin M1 in animal products. Also, the farmers should be educated for the prevention of antibiotic residues in milk.

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