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 sultane, gullac, mukhallebi 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 (Perna 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 investigated 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 mukhallebi, sultane 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 allergy 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 (mukhallebi, sultane, gullac 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).

Gullac is a Ramadan dessert which consists of very thin large dough layers in the milk and rose water, served with pomegranate seeds and walnut. Mukhallebi is a sweet milk pudding dessert and sultane 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 mühallebi, sulac and gulaç similar milky desserts so that they are in the same groups 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 standards and an interpretative result was determined.

Ice cream, mühallebi and sulac samples 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 mL of 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, Belguque) was used for qualitative detection of total antibiotics and sulphonamides in the samples according to manufacturer’s instructions with some pretreatment modifications. The kit 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. Caldicolatus. The milk samples from gullac were pipetted directly on the surface of the agar. The samples which were taken from the mühallebi, 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, ampicilllin 3-4 ng mL⁻¹, amoxicillin 3-4 ng mL⁻¹, coxacillin 10-20 ng mL⁻¹, cloxacinill 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, erytromycin 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, chlorotetracycline 100-200 ng mL⁻¹, chloramphenicol 2500-3500 ng mL⁻¹, bacitracin 1500-3000 ng mL⁻¹, sulphamетazine 100-200 ng mL⁻¹, sulfdiazine 25-100 ng mL⁻¹, sulfdimetoxina 25-100 ng mL⁻¹, sulfamerazine 25-100 ng mL⁻¹, sulflanoxazol 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 mühallebi and sulac, 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 (TFCD, 2002).

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of samples (n)</th>
<th>Aflatoxin M1 positive samples (n)</th>
<th>Aflatoxin Levels Mean±SE (ng kg⁻¹)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice cream</td>
<td>16</td>
<td>6</td>
<td>6.00±4.18</td>
<td>UDL</td>
<td>26.18</td>
</tr>
<tr>
<td>Mühallebi and Sulac</td>
<td>21</td>
<td>15</td>
<td>32.3±11.80</td>
<td>UDL</td>
<td>206.1</td>
</tr>
<tr>
<td>Gullac</td>
<td>10</td>
<td>10</td>
<td>83.5±33.20</td>
<td>12.4</td>
<td>352.9</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>31</td>
<td>34.25±11.51</td>
<td>UDL</td>
<td>352.9</td>
</tr>
</tbody>
</table>

Value is statistically different from Mühallebi-Sulac and Gullac groups (p<0.05). Value is statistically different from Ice cream and Gullac groups (p<0.05).

SE: Standard Error; Min: Minimum; Max: Maximum; UDL: Under the Detection Limit

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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, sultac and gullac. Gullac has the highest milk content as compared to other groups.

Kart et al. (2006) 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 65.8% 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.

REFERENCES


