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Incidence of Some Food borne Pathogens, Heavy Metal Residues and Aflatoxin M1 in Imported Skimmed Milk Powder

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Abstract

Out of 3275 imported skim milk powder packed in 20 Kg paper bags, 95 samples were randomly selected.Milk powder paper bags were imported from USA (n=1315), France (n=940), Denmark (n=555) and Germany (n=465) through Cairo Airport during 2016. The samples were taken as following, 40, 20, 20 and 15 samples from consignments imported from USA, France, Denmark, Germany, respectively. Collected samples were analyzed for the presence of some foodborne pathogens as Salmonella, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*. The samples were also examined for the presence of heavy metal residues and Aflatoxin M_1(AFM1) contamination. The results showed that all samples were free from the examined foodborne pathogens. The heavy metal residues screening revealed that the percentages of presence of lead in the samples imported from USA, France, Denmark and Germany were 22.5, 35, 30 and 26.7% respectively, the percentages of presence of cadmium were 17.5, 20, 25 and 13.3 respectively, the percentages of presence of mercury were 12.5, 15, 10 and 13.5% respectively and AFM1 were detected in 25, 35, 20 and 20% respectively. However, no samples have concentrations exceeding the permissible limits recommended by Egyptian standard. It can be concluded that the examined imported skimmed milk powder samples were free from pathogenic microorganisms and the hazardous metals and Aflatoxin M_1were within the permissible limits and such skimmed milk powder could be judged safe for human consumption.

Keywords

Food borne Pathogens, Heavy Metals, Aflatoxin M_1, Milk Powder

Introduction

Milk powder is one of the most imported dairy products in many developing countries, including Egypt, as it covers the shortage of the fluid milk supply (Aly and Elewa, 2014). It is used in the manufacture of many dairy products such as yoghurt, cheese, frozen desserts, condensed milk, evaporated milk and infant milk formula, in addition it is used as a food ingredient in several value-added foods, such as bakery products and meat products (Salah et al., 2013).

The microbiological hazards associated with milk powder has always been a matter of consideration for the dairy industry despite the high temperature attained in its processing, as faulty processing may be responsible for contamination of milk powder with hazardous foodborne pathogens (Blank et al., 2004). *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and Salmonella have great adverse effects on human health and must be absent in dried milk powder (Pal et al., 2016).

The consumption of milk powder contaminated with heavy metals even with small amount can impose serious risks for human health as gastrointestinal diseases, kidney diseases, heart diseases, teratogenesis, mutagenesis and damage to the nervous system, as the metals that cannot be metabolized as cadmium; lead and mercury persist in the body and exert their toxic effect (Friberg and Elinder, 1988; Järup, 2003).

On the same context, aflatoxins are carcinogenic, teratogenic and mutagenic metabolites mainly produced by Aspergillus flavus and Asergillusparasiticus. Aflatoxin M_1 (AFM1) is a hydroxylated metabolite of aflatoxin $B_1$and can be detected in milk and dairy productsfrom dairy cattle that have ingested feed contaminated with aflatoxin B_1(Diaz et al., 2005). AFM1 is neither affected by storage nor processing and can be detected in dairy products submitted to pasteurization and sterilization process (Pictininet al., 2013).

In Egypt, Cairo airport considered as one of the most important gates for entrance of imported food products, thus strict control measures must be applied to ensure that only safe food are entering the country. Therefore, the current work was planned to investigate the microbiological safety, detection of heavy metals as well as determination of aflatoxin M_1in the examined skim milk powder samples imported via Cairo airport.

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Material and Methods

Collection of Samples

Milk powder 20 Kg paper bags (n=3275) imported from USA (n=1315), France (n=940), Denmark (n=555) and Germany (n=465) through Cairo Airport during 2016 were randomly sampled. 95 samples were taken as following, 40, 20, 20 and 15 samples from consignments imported from USA, France, Denmark, Germany, respectively.

All samples were kept in clean dry containers and transported to Animal Health Research Institute laboratories, Doki, Giza, Egypt to be examined.

Bacteriological examination

Detection of Salmonella was done using the presence/absence method (US FDA, 2011). The suspected isolates were identified according to Forbes et al. (2007).

Detection of *Staphylococcus aureus* was carried out by direct plate count method on Baird Parker agar supplemented with egg yolk tellurite emulsion according to CSN EN ISO 6888-1 (1991). Detection of *Escherichia coli* was carried out on Eosin Methylene Blue (EMB) agar according to Kornacki and Johnson (2001).Detection of *Listeria monocytogenes* was done using Oxford agar as previously described by Rodas-Suarez et al. (2013).

Heavy metals detection

For detection of lead, cadmium and mercury, the skimmed milk powder samples were prepared and analyzed for their heavy metals content as described by Abdelkhaleket al. (2015).

Determination of aflatoxin M_1

Aflatoxin M_1 was determined by enzyme-linked immunosorbent assay (ELISA), using Rida screen AFM1 kits (R-Biopharm, Derm-Stadt, Germany), which contained Microtiter platescoated with specific antibodies to AFM1 as described by Elsayed and Abd El-Fatah (2015).

Results and Discussion

Bacteriological examination

In dried and infant milk formula,microbial pathogensasSalmonella, S. aureus, E. coli and L. monocytogenes are of major concern and must be absent asthese organisms may remain viable in the dried powder, and when the powder is reconstituted and stored at favorable temperature their growth could be resumed (Palet al., 2016). The resultso f the present study showed that all the examined imported skimmed milk powder samples were free from Salmonella, *S. aureus*, *E. coli and L. monocytogenes* and complied with the Egyptian Standard 1648 (Egyptian Standards, 2005).

Absence of pathogenic microorganism from the examined milk powder samples confirm the good hygienic measures that were applied during production, processing, handling and distribution of the product.

The obtained results agree with that obtained by Aly and Elewa (2014), as they did not detect Salmonella in any of their examined samples however, our results regarding *S. aureusare* in disagreement with their results as they detected *Staphylococci* in 66.6% of skimmed milk powders. Rodas-Suarez et al. (2013) isolated *L. monocytogenes* (4.2%) from dry skim milk samples. The presence of Listeria in dry skim milk samples may be caused by cross contamination in the postprocessing stage (Tompkin, 2002).

Heavy metals detection

The interest in metal contamination of food stuffs is increasing, mainly due to the global environmental pollution with heavy metals. The most important heavy metals that have serious consequences on health are lead, cadmium and mercury (Shahriar et al., 2014). Lead is a neurotoxic and of major public health concern as it causes both acute and chronic intoxication and encephalopathy in children (Carl, 1991). In this study, 22.5%, 35%, 30% and 26.7% of the examined samples from USA, France, Denmark and Germany were positive for lead residues with mean concentration of 0.0128, 0.0123, 0.0125 and 0.0152, respectively (Table 1).

Abdelkhaleket al. (2015) reported nearly similar results, as they found that the mean concentration of lead was 0.03mg/Kg in examined skimmed milk powder in Mansoura city, Egypt. While Abdulkhaliqet al. (2012) reported that the mean concentration of lead was 0.002 mg/kg in examined skimmed milk powder in Ramallah City, Palestine. On the other hand, Gasmallaet al. (2013) detected lead in whole milk powder and children dry milk with a mean concentration of 3.3250, and 3.64375 mg/kg respectively, which is higher than our findings. Also, Elbarbary and Hamouda (2015) detected lead in a concentration of 1.61 mg/ kg in examined whole milk powder samples with a minimum of 0.59 and a maximum 3.16 mg/ kg.

One of the main reasons of skimmed milk powder pollution with lead residues is the contamination of original milk used for its manufacturing (Nasef, 2002). Contamination may also occur during manufacturing practices and from the used equipment (Caggianoet al., 2005).

Cadmium is a toxic metal which has adverse effects on the kidney and may cause bone defects and fractures (Järup, 2003). Results recorded in table (2) show that cadmium was present in 17.5, 20, 25 and 13.3% of the examined samples from U.S.A, France, Denmark and Germany with mean concentrations of 0.0213, 0.0153, 0.02 and 0.023, respectively. Nearly similar results were reported by Abdelkhaleket al. (2015). Lower findings were reported by Abdulkhaliqet al. (2012) who detected cadmium with a mean concentration of 0.00001 mg/kg and by O'Keeffeet al. (2001) who could not detect cadmium in any of the examined skimmed milk powder samples. While Gasmallaet al. (2013) and Elbarbary and Hamouda (2015) detected that cadmium levels in milk powder were 4.0625±1.9 and 0.08 to 1.04 mg/kg respectively.

Industrial and agricultural processes are the indirect reasons for the presence of cadmium residues in examined skimmed milk powder samples, as such processes usually lead to increasing the concentrations of heavy metals in air, water, soil and subsequently, these metals reach plants or animals and find their ways into food chain (Ahmad, 2002).

In the present study, mercury was detected in 12.5, 15, 10 and

Country of origin	Examined Samples No.	No. of Positive (%)	Min	Max	Mean ± SE	Comply with Egyptian regulation (0.3 ppm)
USA	40	9 (22.5)	0.011	0.015	0.0128 ±0.00046	100%
France	20	7 (35)	0.011	0.013	0.0123 ±0.00028	100%
Denmark	20	6 (30)	0.012	0.014	0.0125 ±0.00042	100%
Germany	15	4 (26.7)	0.014	0.016	0.0152± 0.00040	100%

Table (1): Lead concentrations (ppm) in the examined skimmed milk powder samples and comparing the detected levels to levels of the existing Egyptian regulation

13.5% of the examined samples from USA, France, Denmark and Germany with mean concentration of 0.0112, 0.013, 0.0105 and 0.015, respectively (Table 3). Higher concentration was reported by Elbarbary and Hamouda (2015) (0.14 ± 0.006 mg/Kg). The presence of mercury in skimmed milk powder may caused by the consumption of polluted feed stuffs and water as well as the excessive use of fungicides and pesticides that contain this metal (Ameret al. 2005).

The permissible limits of heavy metals recommended by Egyptian Standard 7136 (Egyptian Standards, 2010) are 0.3, 0.05 and 0.02 ppm for lead, cadmium and mercury respectively. The maximum concentration of lead, cadmium and mercury in all samples examined in the present study did not exceed the permissible limits and therefore complied with the Egyptian standard.

Determination of aflatoxin M_1

Aflatoxin M_1 is responsible for serious public health hazards among heavily milk consumers especially infants and children. AFM1 is a hepatocarcinogen (Pivaet al., 1995). In Egypt, the incidence of hepato-cellular carcinoma was doubled throughout the past decade (Iyeret al., 2010), that may be attributed to mycotoxin contamination.

Aflatoxin M_1 was detected in 25, 35, 20 and 20% of examined samples from USA, France, Denmark and Germany with mean concentration of 0.0112, 0.012, 0.01325 and 0.013, respectively (Table 4). All samples were below the permissible limit (0.05 ppb) recommended by Egyptian Standard (2010). Higher values were reported by Bonessiet al. (2003), Aly and Elewa (2014), Elsayed and Abd El-Fatah (2015).

Country of origin	Examined samples No.	No. of Positive (%)	Min	Max	Mean ± SE	Comply with Egyptian regulation (0.05 ppm)
USA	40	7 (17.5)	0.017	0.024	0.0213 ±0.0009	100%
France	20	4 (20)	0.013	0.017	0.0153 ±0.0008	100%
Denmark	20	5 (25)	0.019	0.021	0.02 ±0.0004	100%
Germany	15	2 (13.3)	0.019	0.027	0.023 ±0.004	100%

Table (2): Cadmium concentrations (ppm) in the examined skimmed milk powder samples and comparing the detected levels to levels of the existing Egyptian regulation

Country of origin	Examined samples No.	No. of Positive (%)	Min	Max	Mean ± SE	Comply with Egyptian regulation (0.02 ppm)
USA	40	5 (12.5)	0.010	0.012	0.0112T ±0.00037	100%
France	20	3 (15)	0.012	0.014	0.013 ±0.00058	100%
Denmark	20	2 (10)	0.010	0.011	0.0105 ±0.0005	100%
Germany	15	2 (13.3)	0.013	0.017	0.015 ±0.002	100%

Table (3): Mercury concentrations (ppm) in the examined skimmed milk powder samples and comparing the detected levels to levels of the existing Egyptian regulation

Country of origin	Examined samples No.	No. of Positive (%)	Min	Max	Mean ± SE	Comply with Egyptian regulation (0.05 ppb)
USA	40	10 (25)	0.010	0.012	0.0112 ±0.00025	100%
France	20	7 (35)	0.011	0.013	0.012 ±0.00031	100%
Denmark	20	4 (20)T	0.012	0.014	0.01325 ±0.00048	100%
Germany	15	3 (20)	0.010	0.013	0.013 ±0.00058	100%

Table (4): Incidence of aflatoxin M1 (ppb) detection in examined skimmed milk powder samples

Decreasing the daily intake of aflatoxin B_1 contaminated feeding for dairy cattle and implementing a food control system, such as the HACCP system, in the food industries will be beneficial for limiting mycotoxin contamination and decreasing aflatoxin M_1 concentration dairy products as skimmed milk powder (Elsayed and Abd El-Fatah, 2015). In the same context, frequent analytical surveillance by food control agencies is highly recommended to control the incidence of mycotoxin contamination.

Conclusion

It can be concluded that all the examined skimmed milk powder samples imported from different countries were free from pathogenic microorganisms and the hazardous metals and Aflatoxin M_1were within the permissible limits and such skimmed milk powder could be judged safe for human consumption based on the Egyptian Standards.

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