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Occurrence of Ochratoxin A in Milk powder Marketed in Sharkia Governorate

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ABSTRACT

Ochratoxins are a group of mycotoxins produced as secondary metabolites of several fungi, which consists of three members, Ochratoxin A is the most abundant, the most commonly detected member and the most toxic out of the three. A total of 60 random samples of milk powder marketed in Sharkia Governorate, Egypt (20 each of retail size packages (25gm - 100gm), small packages (2kg) and bulk packages (25 kg) were collected and subjected to quantitative detection of Ochratoxin A using immunoaffinty column method after extraction and then reading by VICAM Fluorometer. Out of 20 examined retail size packages samples, 50% were contaminated with Ochratoxin A, the minimum was 2.0 ppb, the maximum was 7.6 ppb with a mean value of 5.14 ± 0.647 ppb, while 65% out of examined small packages were contaminated (3.0 to 8.9 ppb) with a mean value of 6.07 ± 0 .679 ppb, but 80% of bulk packages were contaminated by range value from 3 to 9.9 ppb with an average of 6.51 ppb. All positive samples were within the permissible limits, according to JECFA, 2001, while according to European commission limits, 2001 20%, 25% and 35% of retail size packages, small packages and bulk packages were within the permissible limits, respectively. But according to United States standard and Egyptian limits, 2010, 50%, 35% and 20% were within the permissible limits respectively.

Key words: Milk powder, Ochratoxin A, VICAM Fluorometer

INTRODUCTION

Milk is a key source of nutrients for humans. This is especially significant for infants and children, Milk powder is made by removing water from liquid milk to be not more than 5%. Removing water is necessary to reduce water activity for the prevention of microorganism growth *(1)*. The advantages of dry milk over liquid milk are better keeping quality, less storage space and low shipping costs *(2)*. Dry milk is used to produce such products as yoghurt, cheese; ice-cream and whey powder, large quantities of powder milk have been imported by both general and private sectors from different origins.

Mycotoxins are produced by various strains of fungi, particularly *Penicillium, Aspergillus and Fusarium*, which usually enter the body via ingestion of contaminated foods. Mycotoxins are well known as a worldwide health problem particularly in countries with high ambient temperature and relative humidity typical of tropical countries which favor the growth of fungi in food products, therefore they are potential for the production of mycotoxins *(2-5)*.

Ochratoxins are a group of mycotoxins produced as secondary metabolites by several fungi of the Aspergillus species as *A.ochraceous*. Recently it was shown to be produced commonly by *A.carbonarius* and rarely by the related species *A. niger*, also produced by *Penicillium* species as *P. verrucosum* and *P. nordicum* as they weak organic acids consisting of a derivative of an isocoumarin. Ochratoxins consist of three members; A, B, and C which differ slightly

from each other in their chemical structures. It is the major metabolite both in to occurrence and toxicity. As in other mycotoxins, ochratoxin A can contaminate a wide variety of foods as a result of fungal infection. Ochratoxin A may be present in foods even when the visible mould is not seen. *(6,7)*. Also, mycotoxins are invisible, odorless molecules, and cannot be detected by taste *(8)*.

OTA is a stable compound that is not destroyed by usual heat treatment as cooking and roasting procedure in the process of coffee beans.*(9)*. So it persist during the transformation and processing of contaminated plants *(10,11)*, and constitutes a potential risk for human and animal health *(12)*.

OTA causes carcinogenic, teratogenic, immunotoxic, genotoxic and possibly neurotoxic effects in some laboratory animals *(13)*. Moreover, it is a well-known nephrotoxic agent and has been associated with fatal human kidney disease, referred to as Balkan Endemic Nephropathy and with an increased incidence of tumors of the upper urinary tract *(14)*.

The rumen has a fundamental role in defending cattle and sheep from mycotoxins, and can be considered the first barrier against transfer of mycotoxins from contaminated feeds to milk. The rumen microflora acts as a biological filter, transforming or degrading (as in the case of OTA) mycotoxins into less toxic substances. OTA transfer to milk has been demonstrated in rats and humans, OTA can also be transferred to cow's milk, but only at very low levels except when massive doses are ingested *(15)*.

Therefore, the present study was conducted to determine the level of Ochratoxin A in different types of milk powder collected from Sharkia Governorate, Egypt, to judge its suitability for human consumption as well as using in manufacturing of other dairy products and bakery industry.

MATERIAL AND METHODS

A total of 60 random samples of milk powder (20 each of retail size packages (25 – 100 gm), small packages (2kg) and bulk packages (25 kg) were collected from different supermarkets and local small sweet factories in Sharkia Governorate, Egypt. Samples from bulk packages were represented by 250 grams which was apparently of good condition while another examined samples were collected in its original package.

The detection of OTA residues in collected milk powder sampleswas carried out using immunoaffinity column method for extraction of Ochratoxin A and reading by VICAM Fluorometer in parallel with standard of Ochratoxin A *(16)*.

Preparation of samples

50 gm of the sample and 5 gm NaCL were weighted and placed in blender jar. 100 ml of methanol – water solution(8:2) were added. The sample was blended at high speed for 1 min. and filtrated in a clean vessel.

Extract Dilution

10 ml of filtered extract were poured into a clean vessel, The extract was diluted with 40 ml of purified water and mixed well. The extract was filtered through microfiber filter and the filtrate was collected in a clean vessel.

Column Chromatography

10 ml of diluted extract were passed completely through Ochra Test Affinity Column at rate of about 1-2 drops/ second until air comes through column. 10 ml of mycotoxin wash buffer was passed through the column at a rate of 1-2 drops/ second, followed by passing 10 ml of purified water through the column at a rate of 1-2 drops/ second until air comes through column. Elute affinity column by passing 1.5 ml OchraTest eluting solution through column at a rate of 1 drops/ second and collecting all of the sample elute in a glass cuvette, to be mixed well and the cuvette was placed in a calibrated Fluorometer, Ochratoxin concentration was read and recorded after 60 seconds.

RESULTS

Table 1. Incidence and levels of Ochratoxin A (ppb) in examined milk powder samples (N=20 each)

Types of milk powder	Positive samples		Detection limit (ppb)		Mean ± SE
	No.	%	Min.	Max.	
Retail size packages (25gm - 100gm)	10	50.0	2	7.6	5.14 ± 0.647
Small Packages (2kg)	13	65.0	3	8.9	6.07 ± 0 .679
Bulk Packages (25 kg)	16	80.0	3	9.9	6.51 ± 0.645

Table 2. Frequency distribution of Ochratoxin A in examined milk powder samples in relation to permissible limits of Food Authorities (N=20 each).

Types of milk powder	Detection limit (ppb)						Current ochratoxin regulations					
							JECFA[1] Permissible limit (10 ng/g)		EC Limits[2] Permissible limit (5 ng/g)		US Standards[3] E Standard[4] Permissible limit (Nil)	
	0		2-5		6-10							
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Retail size packages (25 - 100gm)	10	50.0	4	20.0	6	30.0	20	100.0	4	20.0	10	50.0
Small Packages (2kg)	7	35.0	5	25.0	8	40.0	20	100.0	5	25.0	7	35.0
Bulk Packages (25 kg)	4	20.0	7	35.0	9	45.0	20	100.0	7	35.0	4	20.0

1- JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2001)
2- EC Limits (European Commission Regulation, 2001)
3- US Standards (United States Standard)
4-E Standard (EOSQC, 2010)

DISCUSSION

The results given in table (1) revealed that out of 20 examined retail size packages milk powder samples, 10 (50%) were contaminated by Ochratoxin A, the minimum was 2.0 ppb, the maximum was 7.6 ppb and the mean value was 5.14 ± 0.647 ppb, while 13 (65.0%) out of examined small packages were contaminated by Ochratoxin A and the level of contamination was ranged from 3.0 to 8.9 ppb

with a mean value of 6.07 ± 0 .679 ppb, but in bulk packages milk powder samples 16 (80%) were contaminated with a mean value of 6.51 ± 0.645 ppb(3 to 9.9 ppb). Nearly similar findings of small packages were reported by *(17)*. While in case of bulk packages nearly results were detected by ***(18)***. Lower results were detected by ***(19)***, but *(20)* was not found Ochratoxin residues.

These finding were explained why the percentage is low in milk because the Ochratoxin A is rapidly converted into less toxic Ochratoxin α (lacking the phenylalanine moiety) by the fore stomach flora, and only very small amounts of intact Ochratoxin A are absorbed which degraded by rumen protozoa. This effective deactivation explains relative tolerance of ruminants to Ochratoxin A exposure ***(21,22)***.

So to produce high quality milk products, it is essential to prevent feed stuffs from contamination by mycotoxin by good manufacturing and storage practices.

The results listed in table (2) declared that all positive samples of the examined three types were within the permissible limits, according to *(23)*, while according to *(24)* 20%, 25% and 35% of retail size packages, small packages and bulk packages were within the permissible limits respectively, and according to *(25)* 50%, 35% and 20% were within the permissible limits respectively.

Consumption of foods produced from these commodities may pose a potential risk to the health of the infants, since the toxins are resistant to various usual food processing, including heat- treatment ***(26-29)***.

Several epidemiological studies have been conducted in countries in northern Africa, such as Egypt ***(30)***, Algeria, Morocco *(31)* and Tunisia, to establish a causal relationship between OTA and a human nephropathy, and tentatively called chronic interstitial nephropathy (CIN).

Currently, Ochratoxin A is receiving increasing attention for its toxic effects on human health and high incidence in a wide range of food commodities. Although the Ochratoxin A amounts detected were relatively low, the levels may accumulate in the body of humans consuming contaminated food. Ochratoxin A is often not rapidly removed from the body and it is frequently found in human blood due to its long elimination half-life (about 35 days in serum), as a consequence of its binding to plasma proteins, its enterohepatic circulation and its reabsorption from urine ***(32)***. This makes of Ochratoxin A the most detected mycotoxin in human blood all over the world ***(33)***.

Chances of mycotoxins production are very small when dairy products are kept at refrigeration temperature and good hygiene practice is very important to fight mould spoilage. Because air is generally an effective vehicle for distribution of mould, filteration of air and even the practice of clean room technique had been introduced in some places. Vacuum packaging or modified atmosphere packaging was used to inhibit mold growth, and application of chemical inhibitors on wrapping and product surface was also used ***(34)***.

In conclusion, the results revealed the presence of Ochratoxin in the examined samples which considered as risk factor in dairy production. Consequently, more restriction and preventive measures should be taken in milk herds, milk production and dairy factories in respect to quality control, sanitation and health care. So to produce high quality milk, it is essential to keep raw materials from contamination. Mycotoxin concentrations in raw materials can be reduced by good manufacturing practice and good storage practices. All efforts should be made to ensure that the toxins are below permissible limits. Periodic testing of food for mycotoxins is important to control occurrence of the toxin in the food chain. Since it is difficult to remove the mycotoxin once formed, the best way of control is prevention. Mycotoxin control requires proactive HACCP-based strategies to prevent fungal growth.

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الملخص العربى

تواجد الاوكراتوكسين A فى اللبن المجفف المسوق فى محافظة الشرقية

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الاوكراتوكسينات هي مجموعة من السموم الفطرية المنتجة والمركبات الثانوية من قبل عديد من الفطريات، والتي تتألف من ثلاثة مجموعات، الأوكراتوكسين A هي الأكثر وفرة و شيوعا و سمية من الثلاثة. وقد تم تجميع ٦٠ عينة عشوائية من مسحوق اللبن المجفف من محافظة الشرقية، بمصر (٢٠ عينة من كل من عبوات صغيرة الحجم (-١٠٠٢٥جم) وعبوات متوسطة الحجم ٢ كجم و عبوات كبيرة الحجم ٢٥ كجم) لإجراء الكشف الكمي للأوكراتوكسينات بواسطة جهاز VICAM Fluorometer .و أكدت النتائج انه من بين ٢٠ عينة صغيرة التي تم فحصها كانت ملوثة بنسبة ٥٠٪ مع الأوكراتوكسين A ، وكان الحد الأدنى هو ٢ جزء في البليون، وكان الحد الأقصى ٧.٦ جزء في البليون وكان بمتوسط قدره ٥.١٤ ± ٠.٦٤٧ جزء في البليون . في حين أن ٦٥٪ من مجموعات العينات زنه ٢ كجم كانت ملوثة بمستويات تراوحت ٣- ٨،٩ جزء في البليون بمتوسط قدره ٦.٠٧ ± ٠،٦٧٩ جزء في البليون، بينما ٨٠٪ من العينات حجم ٢٥ كجم كانت ملوثة بقيم تراوحت بين ٣- ٩،٩ جزء في البليون بمتوسط قدره ٦.٥١ ± ٠.٦٤٥ جزء في البليون. كما أظهرت النتائج أن جميع العينات الإيجابية لم تتعدى الحد المسموح به لـ JECFA -2001. بينما وجد أن ٢٠٪، ٢٥٪ و ٣٥٪ من العينات الصغيرة و المتوسطة و الكبيرة الحجم كانت في نطاق الحد المسموح به كما هو مقر بالمفوضية الأوروبية عام ٢٠٠١على التوالي فى حين أن ٥٠٪، ٣٥٪ و ٢٠٪ من العينات السابق ذكرها كانت في نطاق الحد المسموح به في حدود القياسات الأمريكية والمصرية لسنة ٢٠١٠ على التوالي.