

Prevalence of ochratoxin in small and large scale produced roomy cheese in Sharkia Governorate

Awad, E.I. ¹Abdelfattah M. E. ², Abdelkaliek, A.A. ² and El-diasty, E.M. ³

¹Food control department, Faculty of Vet .Med. Zagazig University, Egypt

² Food Hygiene Unit and ³Mycology Department, Animal Health Research Institute, Dokki – Giza –Egypt

Emanhoda2003@yahoo.com

Abstract: A total of 40 random samples of roomy cheese (20 each of small and large scale production) were collected from different supermarkets in Sharkia Governorate, Egypt. The samples were subjected to quantitative detection of Ochratoxin A by immunoaffinity column method after extraction of ochratoxin A and reading by VICAM fluorometer. Out of examined 20 small scale manufactured roomy cheese samples, 12 (60%) were contaminated by ochratoxin A, the minimum detected level was 2.0 ppb, the maximum was 7.0 ppb and the mean value was 3.67 ± 0.22 ppb while only 6 (30%) out of examined large scale manufactured roomy cheese samples were contaminated by ochratoxin A. The level of contamination detected ranged from 2.0 ppb to 5.0 ppb with a mean value of 3.92 ± 0.30 ppb. All positive samples are more than the permissible limits, according to United States standard, European commission limits and Egyptian limits (Permissible Limit Nil). While according to limit established by JECFA (Joint FAO/WHO Expert Committee on Food Additives) all positive samples (18) below the permissible limit (10ng/g). The results indicated that large scale produced roomy cheese had low incidence of ochratoxin A. This may be due to vacuum packaging, products are kept at refrigeration temperature, and good hygiene practice during ripening process.

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1. Introduction

Cheese is known to be of great nutritional value for human consumption as its fat and protein have a high biological value and contains all essential fatty and amino acids. Also it is a source of vitamins and minerals. However, cheese is very susceptible to mould growth and is normally kept under refrigeration.

Roomy cheese is fermented hard cheese manufactured from raw cow and buffalo's milk in Egypt. Environmental conditions prevailing during cheese ripening, combined with the composition of the cheese often create possibilities for extensive development of mould on cheeses surfaces, which reduces quality. As a result of mould growth mycotoxins may be produced in cheese, rendering it unfit for human consumption (Pitt and Hocking, 2009). Factory air was identified as an important source of contamination (Kure *et al.*, 2004, 2008). In Turkish Kuflu cheese, *Penicillium* species, including *P. commune*, *P. roqueforti* and *P. verrucosum*, were also identified as the dominant spoilage organisms, being isolated from over 70% of samples (Hayaloglu and Kirbag, 2007).

Ochratoxins are a group of mycotoxins produced as secondary metabolites by several fungi of the *Aspergillus* species as *A. ochraceus*. Recently it was shown to be produced commonly by *A. carbonarius* and rarely by

the related species *A. niger*. However, Ochratoxins is also produced by *Penicillium* species as *P. verrucosum* and *P. nordicum* and are weak organic acids consisting of a derivative of an isocoumarin (Fig. 1). The family of ochratoxins consists of three members, A, B, and C which differ slightly from each other in chemical structures. These differences, however, have marked effects on their respective toxic potentials. Ochratoxin A is the most abundant and hence the most commonly detected member but is also the most toxic of the three. It is a potent toxin affecting mainly the kidney. 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 (FAO/WHO, 2006; Michael and Larry, 2006).

There is considerable information on the natural occurrence of OTA in human foods. Chiara *et al.* (2008) reported the occurrence of ochratoxin A in blue cheeses for the first time. Ochratoxin A is found in infant formulae and baby foods (Terken *et al.* 2007), dairy cows milk (Coffey *et al.*, 2009; Pattono *et al.*, 2011), powder milk and soft cheese (Hassan and Hamed, 2001), meat and edible organs (Gareis and Scheuer, 2000).

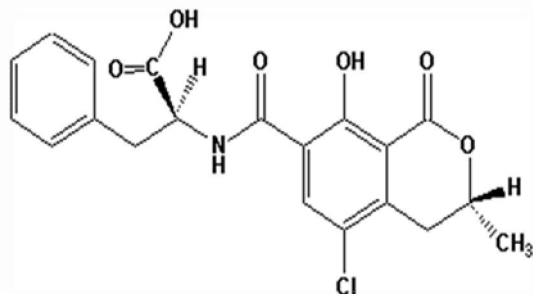


Figure (1): Structure of Ochratoxin A (OA).

OTA is a nephrotoxic, teratogenic and carcinogenic compound and may also cause immunotoxic effects (Kuiper-Goodman, 1991; Pestka and Bondy, 1994 and Prelusky *et al.*, 1994). Due to its nephrotoxicity, OTA has been regarded as an important factor for human endemic nephropathy in the Balkan area (Petkova-Bocharova and Castegnaro, 1991; Fuchs *et al.*, 1991; Beardall and Miller, 1994), although the evidence is not unambiguous (Plestina, 1996; JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2001).

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, filtration 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 (Sorhaug, 2011).

Therefore the present study was conducted to compare between the prevalence of ochratoxin A in small and large scale produced roomy cheese in Egypt.

2. Materials and Methods

A total of 40 random samples of roomy cheese (20 each of small and large scale) were collected from different supermarkets in Sharkia Governorate, Egypt. Small scale sample were represented by 250 grams which was apparently of good condition while large scale produced samples were collected in its original package.

The detection of OTA residues in collected cheese takeplace using immunoaffinity column method for extraction of ochratoxin A and reading by VICAM fluorometer in parallel with standard of ochratoxin A (Hansen, 1993).

Preparation of samples:

Weigh 50 grams of ground sample and place

in blender jar. Add to jar 100 ml of methanol - water solution. The sample blended at high speed for 1 min. Filtrate mixture in clean vessel.

Extract Dilution:

Pour 10 ml filtered extract into a clean vessel, dilute extract with 40 ml of purified water and mix well. Filter extract through microfibre filter and collect filtrate in a clean vessel.

Column Chromatography:

Pass 10 ml diluted extract completely through ochraTest affinity column at rate of about 1-2 drops/ second until air comes through column.

Pass 10 ml of mycotoxin wash buffer through the column at a rate of 1-2 drops/ second. Pass 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. Mix well and place cuvette in a calibrated fluorometer, read ochratoxin concentration after 60 seconds.

Table (1): Incidence of Ochratoxin A (ppb) in examined roomy cheese samples (n= 20 each)

Type of cheese	Positive samples		Range (ppb)		Mean of level \pm SE(ppb)
	No.	%	Min	Max	
Small scale	12	60	2.0	7.0	3.67 \pm 0.22
Large scale	6	30	2.0	5.0	3.92 \pm 0.30

The results given in table (1) revealed that out of 20 examined small scale manufactured roomy cheese samples, 12(60%) were contaminated by ochratoxin A, the minimum was 2.0 ppb, the maximum was 7.0 ppb and the mean value was 3.67 \pm 0.22 ppb while only 6 (30%) out of examined large scale manufactured roomy cheese samples were contaminated by ochratoxin A and the level of contamination was ranged from 2.0 to 5.0 ppb with a mean value of 3.92 \pm 0.30 ppb. Lower findings were recorded by El-Sawi *et al.*, (1994) and Chiara and Juliano (2007) while Martins *et al.*, (1995) could not detect ochratoxin in any of examined low fat cheese samples. Hassan and Hammad (2001) detected Ochratoxin A residue in one milk powder samples (3%) at levels of (2.0ppb).Kokkonen *et al.*, (2005) analyzed nine mycotoxins in cheese matrix. The method involves liquid extraction followed by high performance liquid chromatographic separation and mass spectrometric detection of the analyses, and allows the determination of aflatoxins B₁, B₂, G₁, G₂ and M₁, ochratoxin A, mycophenolic acid, penicillic acid and roquefortine C simultaneously and found that

none of the examined cheese samples were contaminated by ochratoxin A. **Chiara et al., (2008)** recorded the occurrence of ochratoxin A in blue cheeses for the first time. The development of an accurate and reliable procedure for the extraction of OTA from cheese, as well as the availability of a new sensitive HPLC-FLD method, has allowed to determine ochratoxin A in complex matrices such as cheeses, even at very low levels (LOD in cheese: 0.02 µg/kg). A good linearity for the OTA concentration, between 0.2 and 2.5 µg/kg, was obtained and no matrix effect was observed in the same concentration range. The mean recovery for OTA was 97%, while the average RSD was 3%, within a spiking

range of 0.5–2.0 µg/kg. Although the OTA contamination levels found in blue cheeses were very low, occurrence of ochratoxin A in such products opens a new issue for risk assessment and quality control, as far as finding the origin of the OTA contamination and ways to prevent it. **Amin et al., (2009)** reported that Ochratoxin A was detected in 1 out of 5 (20%) of the dairy Cattle milk samples concentration 2.73 µg/L. **Pattono et al., (2011)** investigated the presence of OTA in sample of conventional milk and found that three samples were positive for OTA with amount ranging from 0.07- 0.11 ppb.

Table (2): Frequency distribution of total ochratoxin A residues in the examined samples In relation to permissible limits of Food Authorities (n=20 each)

	Range of detection (ppb)						JECFA ¹ Permissible Limit (10 ng/g)		US standard ² EC limits ³ E standard ⁴ Permissible Limit (Nil)	
	0*		2 - 5		6 -10		N0.	%	N0.	%
	N0.	%	N0.	%	N0.	%				
Small scale	8	40	10	50	2	10	20	100	8	40
Large scale	14	70	6	30	0	0	20	100	14	70

1-JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2001)

2: United States standard

3: European Commission Regulation (2001)

4: EOSQC (2010).

The results listed in table (2) declared that all positive samples were exceeding the permissible limits, according to United States standard, European commission limits and Egyptian limits (Permissible Limit Nil). While according to limit established by **JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2001)** all positive samples (18) below the permissible limit (10ng/g). In previous studies Samples of organic cow's milk, conventional cow's milk, and cow's milk-based infant formulas were analyzed for the occurrence of ochratoxin A by means of an HPLC method. The detection limit was 10 ng/L. Ochratoxin A was detected in 6 out of 40 conventional cow's milk samples (range 11-58 ng/L), and in 5 out of 47 organic milk samples (range 15-28 ng/L). No ochratoxin A was detected in any of the 20 infant formula samples. The ochratoxin A levels in cow's milk found in this investigation are sufficient to cause a higher intake of ochratoxin A than the suggested TDI of 5 ng/kg B. w/day (**Skaug, 1999**). Ochratoxin A might be produced on the surface of cheese by *penicillia* during the ripening and moulds are important in dairy products because they can indicate spoilage, can produce mycotoxins. Hence, mould growth on dairy products can result in economic losses because of spoilage also may cause public health

concerns if mycotoxins are produce (**Tornadijo, 1998**). **Canadian Grain Commission (2011)** mentioned that European Union sited limits for ochratoxin A for unprocessed cereals as 5ppb and for all products made from unprocessed cereals intended for direct human consumption 3ppb.

In contrast, no regulation for OTA in milk exists (**Boudra, et al., 2007**). Human intake and absorption of OTA were confirmed through the detection of OTA residues in human blood serum, milk and kidney (**Assaf et al., 2004**). OTA was last evaluated by JECFA, 2001 when it concluded that OTA possesses carcinogenic, nephrotoxic, teratogenic, immunotoxic and possibly neurotoxic properties. The SCF estimated that the mean dietary intake ranged from 0.7 to 4.6 ng/kg B.W per day. By combining the average OTA contamination levels in food which is to be consumed with 95% of probability, the JECFA estimated a dietary exposure of approximately 90 ng/kg BW per week corresponding to about 13 ng/kg BW per day.

In conclusion, the results revealed the presence of ochratoxin A in the examined roomy cheese samples in detectable limits 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. Also the obtained results indicated that large scale produced roomy cheese had low incidence of ochratoxin A due to vacuum packaging, products are kept at refrigeration temperature, and good hygiene practice during ripening process.

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