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MONITORING OF AFLATOXIN M_1 IN SOME DAIRY PRODUCTS IN LOCAL MARKET OF ALEXANDRIA, EGYPT: ATTEMPTS FOR DETOXIFICATION

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ABSTRACT

Aflatoxins are carcinogenic and toxic metabolites produced by a variety of molds. Aflatoxin M_1 (AFM_1) accumulates in animal livers and it is excreted in milk, which can be consumed by human. The present study was undertaken aiming at examining the dairy milk products for their any contamination of AFM_1 in Alexandria local market, Egypt, as well as conducting trials for elimination of AFM_1 contamination by using ozone gas and gamma radiation. Levels of AFM_1 contamination (0.001 to 0.06 □g/l) were ranged from in examined milk and dairy products. The maximum levels were contamination in raw and pasteurized milk while minimum levels were detected in infant milk. The result indicated that yoghurt processing decreases or even eliminates the presence of AFM_1, which may be attributed to some factors such as low pH, formation of organic acids or other fermented by-products. Treatment of contaminated liquid milk by ozone gas resulted in a decrease or even loss in the contents of AFM_1. However, ozone treatment for 10 minutes was the effective interval for complete elimination of contamination, but resulted in coagulates milk protein and changed the odor. Thus, it can be stated that ozone gas was a non-convenient method for complete detoxification of milk and dairy products. Treatment by gamma radiation (with a dose of 10 kGy) was found to be more suitable as a detoxification procedure than ozone treatment as there were no change in properties of milk upon treatment.

Keywords: Aflatoxin M_1, Detoxifecation, Milk, Milk product.

INTRODUCTION

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus* and *Aspergillums parasiticus* (Egmond, 1989). Crops may be contaminated by one or more of the four following sub-types of aflatoxin: B_1, B_2, G_1 and G_2. Aflatoxins B_1 is the most toxic and frequently detected form, Aflatoxins have been implicated in human health disorders including hepatocellular carcinoma, aflatoxicosis, Reye's syndrome and chronic hepatitis. Animals are exposed to aflatoxins by consumption of foods that are contaminated by aflatoxin-producing fungal strains during growth, harvest or storage. When cows are fed with contaminated food (Schlatter, 1990, Whitlow & Hagler, 2005 and Ajoy & Priyanka, 2010). Aflatoxins can occur in approximately 20 related fungal metabolites and contaminate a wide range of important commodities, including cereals, nuts, spices, figs dried

fruit and other foods and feeds (abdelhamid,1983,1985 and 1990 and abdelhamid et al ., 1996).

Aflatoxin B_1 (AFB_1) is converted by hydroxylation to aflatoxin M_1 (AFM_1) which accumulates in animal livers, and is excreted in urine and milk (Whitlow & Hagler, 2005). AFM_1 which is subsequently secreted in the milk of lactating cows is quite stable to normal milk processing methods such as pasteurization and may persist into final products for human consumption. The amount of toxins excreted as AFM_1, as a percentage of AFB_1 in feed, is usually 1-3%, but values as high as 6% have been reported (Jouany & Diaz, 2005). Once AFB_1 is absorbed into the cow's body, the clearance of AFM_1 in milk may take 5 to 7 days depending on the amount and duration of the AFB_1 consumption (Whitlow & Hagler, 2005).

The Codex Committee on Food Additives and Contaminants reported the carcinogenic potency of AFM_1 in sensitive species to be one order of magnitude less than that of AFB_1 (Lafont, *et al*, 1989). AFM_1 may occur in animal organs and tissues, e.g. kidneys, and in animal products, e.g. milk, and other dairy products. Sources of aflatoxin contamination in animal feedstuffs may vary geographically. Many feeds may contain aflatoxins but the most important sources are meals of groundnut, cottonseed and maize. Contamination of agricultural crops with aflatoxins is a worldwide problem that occurs where both climatic and technological conditions stimulate aflatoxin formation (SCF, 1994 and Galvano *et al*, 1996).

The Scientific Committee for Food (SCF) concluded that there is sufficient evidence that AFM_1 is a genotoxic carcinogen; its carcinogenic potency is estimated to be approximately 10 times lower than AFB_1. However, because the intakes of milk and milk products by humans are of considerable amounts, particularly among infants and young children, the risks from AFM_1 exposure need careful consideration (FAO, 1997). Global regulations of AFM_1 contamination in milk are varied from one country to the other. It should be less than 0.05 µg/kg in EU, Switzerland, Austria, France, China, Turkey, Japan, Mexico, Thailand, Argentina, and Honduras; less than 0.50 µg/kg in US, Bulgaria; less than 1.0 µg/kg and 0.0 µg/kg in Egypt, Rumania (FAO, 1997).

Because of the serious health hazards that may be associated with exposure to AFM_1, the present study was undertaken aiming at determining the contamination levels of AFM_1 in milk samples and dairy products available for sale in Alexandria, Egypt; assessing the effect of milk processing on AFM_1 content e.g.; manufacturing of soft white cheese, hard white cheese and yoghurt; conducting trials to eliminate AFM_1 contamination in order to reduce the accompanied risk; e.g. using ozone (O_3) gas and gamma (γ) radiation; and finally drawing a conclusive recommendation for healthy use.

MATERIALS AND METHSODS

Chemicals. All reagents, chemicals and solvents were of analytical HPLC grade, provided via Carlo Erba (Milan, Italy). Deionized water was purified by MilliQ system (Waters, Milford, MA, USA). The immunoaffinity columns AflaM_1test were purchased from VICAM (USA). The AFM_1 standard used was obtained from Sigma- Aldrich (Saint Louis, MO, USA, Product Code A-6428, 50 mg) as purified crystalline AFM_1.

Monitoring of AFM_1 in milk and dairy Products. A total of 210 samples (raw milk, pasteurized milk, UHT milk, concentrated milk, infant's milk, milk powdered, crude milk, yoghurt, kareish cheese, soft white cheese, hard white cheese, processed cheese, ice cream, labneh, butter and cheese whey) were analyzed for AFM_1. All samples were obtained from different supermarkets in Alexandria city, North Egypt during 2012-2013, and transported to the laboratory in an ice packed box. The samples were stored at -20 °C in deep-freezer until being analyzed. AFM_1 was determined through a combined cleanup process with immunoaffinity columns and HPLC (Agilent 1200 series, USA) determination (AOAC Official Method 2000 and Manetta *et al*, 2005).

Effect of processing methods on AFM_1 content. Manufacture of soft and hard white cheese with different concentration of AFM_1 was carried out using the traditional method of soft white cheese manufacture according to El-Gawad, 2009 and Abd El-Salam *et al*., 2011. Manufacture of yoghurt with different concentration of AFM_1 was carried out using the traditional method of yoghurt manufacturing (Baraka *et al*., 2011).

Detoxification of milk samples.

1. **Effect of using Ozone (O_3) gas on AFM_1 content.** Ozonation of samples was carried out similarly to application of ozonation in food staffs according to (**Proctor *et al*., 2004 and Inan *et al*, 2007),** using O_3 generator (1-FM-300 Mini generator, USA). Ozone was delivered on site upon demand, at the concentration of 200 mg per hour on ambient air.
2. **Effect of (γ) radiation treatment on AFM_1.** Radiation of samples was carried out similar to radiation of other food staffs according to (**Iqbal *et al*., 2012**) using radiation unit (3500 Norcontrol AS, Vinderen, Oslo 3, Norway).
3. **Detoxification evaluation/ Screening for AFM_1.** Milk samples were further checked after detoxification procedures for the presence of any content of AFM_1, according to **(Kokkonen & Jestoi, 2009**) using GC/MS (Agilent GC/MS system 7000 Series Triple Quadrupole, USA)
4. **Assessment of milk properties.** Milk properties were examined after detoxification procedures. All samples were dried at -50°C, using freeze-dryer (Labconlo Freeze Dryer; Free Zone 6 Liter Benchtop Freeze Dry System with Stoppering Tray Dryer). Nutritional factors were analyzed as follows; calories according to United States Department of (**Agriculture, 1975**); fats according (**AOAC, 2005**); proteins according to (**Barbano *et al*, 1991)**; carbohydrates according to (**AOAC, 1998**); calcium and phosphor

according to AOAC, 2005; and vitamin $B_1$and vitamin B_2 according to (**AOAC, 2001**). The resulted data were investigated for any change in the milk properties.

RESULTS AND DISCUSSION

Occurrence of AFM_1 in samples of milk and dairy products is illustrated in Table 1. The maximum detected level was 0.06µg/l in pasteurized milk; 0.05µg/l in powdered milk and 0.041µg/kg in processed cheese. The mean detection levels varied from 0.006µg/l in infant milk to 0.056µg/l in pasteurized milk. AFM_1 contamination was detected in percentages of 37.5, 33.3, 33.3, 16.6, 37.3, 54.5, 28.5, 26.6, 29.4, 44.4, 25, 33.3, 42.1, 30.7, 25, and 23.08, in raw milk, crude milk, powdered milk, infant milk, pasteurized milk, UHT milk, concentrated milk, yoghurt, processed cheese, soft cheese, hard cheese, kareish cheese, ice cream, labneh, butter and cheese whey, respectively.

Table 1 : Occurrence of AFM_1 in milk and dairy products in samples collected from the local markets in Alexandria.

Dairy products	Samples tested; n	Positive samples, n (%)	Range (µg/l or µg/Kg)	Mean ±SE* (µg/l or µg/Kg)
Raw milk	16	6 (37.5)	0.02 - 0.06	0.04±0.01
Crude milk	6	2 (33.3)	0.019 - 0.017	0.018±0.001
Powdered milk	9	3 (33.3)	0.03 - 0.05	0.0433±0.0068
Infant milk	12	2 (16.6)	0.001 - 0.011	0.006±0.005
Pasteurized milk	8	3 (37.3)	0.05 - 0.06	0.056±0.004
UHT milk	11	6 (54.5)	0.01 - 0.027	0.018±0.003
Concentrated milk	7	2 (28.5)	0.013- 0.015	0.014±0.001
Yoghurt	15	4 (26.6)	0.01 - 0.02	0.015±0.003
Processed cheese	17	5 (29.4)	0.021- 0.041	0.027±0.004
Soft cheese	27	12 (44.4)	0.001 - 0.04	0.025±0.004
Hard cheese	16	4 (25)	0.011- 0.032	0.021±0.005
Kareish cheese	9	3 (33.3)	0.01 - 0.04	0.027±0.009
Ice cream	19	8 (42.11)	0.012 - 0.031	0.021±0.002
Labneh	13	4 (30.7)	0.012 - 0.029	0.021±0.002
Butter	12	3(25)	0.013 - 0.027	0.019±0.005
Whey	13	3(23.08)	0.021 - 0.03	0.025±0.005

***SE; Standard Error, figures are presented in the form of means ± SE.**

The detected levels of AFM_1 in milk and dairy products were compared to the exceeding regulation of US, EU and Egypt (Table 2). All AFM_1 levels detected in raw milk, pasteurized milk, powdered milk, UHT milk, infant milk, crude milk, concentrated milk, yoghurt, processed cheese, soft cheese, hard cheese, kareish cheese, whey, ice cream, labneh and butter were not exceeding the US regulations (0.5 µg/l or µg/Kg), while some samples of raw, pasteurized, and powdered milk were exceeding the EU regulation (0.05 µg/l or µg/Kg) and Egyptian regulations (0.05 µg/l or µg/Kg), with percentage 3%, 2% and 3%, respectively (Table 2). (Abo-zeid *et al.*, 1996) monitored 30 samples of hard

cheese and 28 samples of skim milk-soft cheese for mycotoxins' contamination and results revealed the presence of AFM_1, AFB_2, and AFG_2 at percentages of 3.3, 17.9, and 20, respectively. (Balata and Bahout, 1996), reported that in 24 samples of raw milk collected from camels, AFM_1 was detected in 25% of camel's milk samples, with a mean value of 0.55 µg/L. A study was done by (El-Seadawy *et al.*, 2000) on 200 samples of raw milk products (raw milk, Domiati cheese, processed cheese and yoghurt; 50 samples each) collected from supermarkets to study its bacterial and fungal contamination. AFM_1 was detected in 36 samples (18%) with a level ranged from 10 to 820 ng/kg. Domiati cheese had a relatively higher concentration of AFM_1. In Ismailia, Egypt, (Motawee *et al.*, 2009), detected AFM_1 in milk samples collected from camel, goat, cow and buffalo species, in 80%, 74%, 66% and 52% of samples, respectively. Levels were below the EU maximum levels of AFM_1; less than 50ng/L. In other study in Alexandria,(Amer and Ibrahim, 2010), analyzed 50 raw milk samples and 150 soft, hard and processed cheese samples (50 of each) and reported AFM_1 contamination in 19 samples of raw cow milk, 20 samples of soft cheese, 19 samples of hard cheese and 11 samples of processed cheese. (El Sayed *et al.*, 2011) studied the presence of AFM_1 in 70 samples of the Egyptian style white soft cheese and results revealed that 4 out of 15 Kariesh, 7 out of 30 Domiati, 3 out of 15 Tallaga and 2 out of 10 Feta cheese samples were positive for the presence of AFM_1. The highest and lowest aflatoxin concentrations were 0.4 and 0.1 □g /kg of the cheese. In Cairo, (Mohsen *et al.*, 2011) reported the presence of AFM_1 in 54.6 % of 141 tested milk product samples. The range of AFM_1 was 3.41-137 ng/l, for pasteurized milk, 6.28-67.4 ng/l for powdered milk, 9.70-89.3 ng/l for yogurt, and 7.14-122 ng/kg for Feta cheese.

Table (2): Comparing the detected levels of AFM_1 (µg/l) in samples of milk and dairy products to levels of the existing regulations.

Dairy product	Positive samples	Exceeding Egyptian regulations (0.05 µg/l or µg/Kg)		Exceeding EU regulations (0.05 µg/l or µg/Kg)		Exceeding US regulations (0.50 µg/l or µg/Kg)	
		N (%)	Range	N (%)	Range	N (%)	Range
Raw milk	6	3 (18.8)	0.05 - 0.06	3 (18.8)	0.05 - 0.06	---	---
Crude milk	2	---	---	---	---	---	---
Powdered milk	3	2 (22.2)	0.05	2 (22.2)	0.05	---	---
Infant milk	2	---	---	---	---	---	---
Pasteurized milk	3	3 (100)	0.05 - 0.06	3 (100)	0.05 - 0.06	---	---
UHT milk	6	---	---	---	---	---	---
Concentrated milk	2	---	---	---	---	---	---
Yoghurt	4	---	---	---	---	---	---
Processed cheese	5	---	---	---	---	---	---
Soft cheese	12	---	---	---	---	---	---
Hard cheese	4	---	---	---	---	---	---
Kareish cheese	8	---	---	---	---	---	---
Ice cream	3	---	---	---	---	---	---
Labneh	4	---	---	---	---	---	---
Butter	3	---	---	---	---	---	---
Whey	3	---	---	---	---	---	---

Effect of manufacturing on AFM_1 content.

1. Effect of manufacturing of soft white cheese on AFM_1 content.

Effect of processing methods on AFM_1 content in soft white cheese is shown in Table 3. The mean concentration values of AFM_1 in whey and cheese were 0.23 µg/l & 0.92 µg/Kg, 0.11 µg/l & 0.45 µg/Kg and 0.021 µg/l & 0.088 µg/Kg in contaminated milk samples with three concentrations of 0.5, 0.25 and 0.05 µg/l respectively. The mean concentration of toxin in cheese was 4.1fold more than that in whey and 1.77 fold more than that in milk. The mean concentration of toxin in whey decreased than that of milk. A statistical significant ($p<0.05$) difference was shown between the different concentrations of AFM_1 (0.5, 0.25 and 0.05 µg/l) after soft white cheese manufacturing. AFM_1 is associated with the protein fraction of milk, and hence it is present in cheese approximately 3–5 fold over that in milk. Studies on the fate of AFM_1 in cheese whey processing found that although the AFM_1 has a low molecular weight close to lactose, but does not permeate through the filters as lactose in UHT processing and exhibits a preference for the retentiveness (Mendonca & Vaenancio 2005). (Govaris *et al.*, 2001), reported that the affinity of AFM_1 for caseins is higher when compared to its affinity for serum proteins. Some previous studies exhibit contradictory data on the behavior of AFM_1 during cheese making, found that AFM_1 distribution during cheese making has a reduction of about 60% compared to the milk (Lopez *et al.*, 2001). Our results are in agreement with that observed by (Dosako *et al*., 1980) in other kind of cheese. They reported 3.9-4.4 folds increasing of AFM_1 concentration in Telemes cheese compared with the cheese milk, using HPLC system for determination and quantification of AFM_1. They attributed their results to the reason of AFM_1 being mainly soluble in the aqueous phase of milk and adsorbed to casein particles.

In another study on the distribution of AFM_1 in Camembert cheese processing, the results showed that AFM_1 concentration in cheese was higher than that in cheese milk (Fremy *et al.*, 1990). A study on the contamination of Ewe's cheese milk, curd and cheese with AFM_1 in Feta cheese processing showed that the level of AFM_1 in curd was higher than that of milk and cheese (Grigoradou *et al.*, 2005). In another study, the mean concentrations of toxin in curd and cheese were reported to be 3.12 and 3.65-fold more than that in whey and 1.68 and 1.80 fold more than that in cheese milk, respectively (Kamkar, 2005).

2. Effect of manufacturing of hard white cheese on AFM_1 content.

Effect of manufacturing on concentrations of AFM_1 in hard white cheese is shown in Table 4. By using contaminated milk with three concentrations 0.5, 0.25 and 0.05 µg/l, the mean concentration of toxin in soft cheese and hard cheese was 1.63 and 2.4 folds more than that in cheese milk, respectively. Statistical significant differences between concentrations of AFM_1 in cheese milk and soft and hard cheese after manufacturing were detected at $P< 0.05$.

Table 3: Contents of AFM_1 in cheese and whey samples after soft cheese manufacturing.

Liquid milk with conc. of AFM_1 (µg/l or µg/Kg)	In cheese*			In whey*		
	Range (Min-max) (µg/Kg)	Mean ±SE (µg/Kg)	Conc.(µg/l) Increase	Range (Min-max) (µg/l)	Mean ±SE (µg/l)	%Conc. Decrease
(0.5)	0.99-0.87	0.92±0.0205	1.84	0.24-0.23	0.23±0.0032	54
(0.25)	0.49-0.41	0.45±0.0141	1.80	0.12-0.10	0.11±0.0032	56
(0.05)	0.092-0.082	0.088±0.0015	1.76	0.023-0.020	0.021±0.0005	58

*** Contents of AFM_1after processing were obtained using 5 replicates, SE; Standard Error, figures are presented in the form of means ± SE.**

Our results are in agreement with those observed by (Yousef & Marth, 1989, and JECFA, 2001) who reported that AFM_1 seems to be predominantly associated with casein, so that cheese curd contains a higher concentration than whey. Association of AFM_1 with casein can be expressed as an enrichment factor (EF) for AFM_1 during cheese-making. Studies showed that the concentration of AFM_1 is about 3 folds higher in many soft cheeses and about 5 folds higher in hard cheeses than in milk. Some studies demonstrated that cheese ripening and proteolysis of casein increase the recovery of AFM_1 from naturally contaminated milk; proteolysis may affect hydrophobic regions on casein associated molecules releasing of AFM_1 (Yousef & Marth, 1989, and JECFA, 2001).

3. Effect of manufacturing of yoghurt on AFM_1 content.

Effect of manufacturing of yoghurt on AFM_1 content is shown in Table **5**. Yoghurt was manufactured by using milk contaminated with AFM_1 in three concentrations; 0.5, 0.25 and 0.05 µg/l. The mean concentrations of toxin in yoghurt, for the three concentrations respectively, were decreased with percentage of 6, 18, 20, 26%, 16, 20, 24, 28 % and 20, 28, 64, 68%. at zero time (fresh yoghurt), and after one, two, and three days, respectively. The decreases in AFM_1 contents were associated with gradual decreases in pH values. Statistical significant differences $p< 0.05$. between decreases in concentrations of AFM1 were detected after yoghurt manufacturing The noted decrease in content with AFM_1 with yoghurt manufacturing is in agreement with (Govaris *et al.*, 2002). Other studies reported no influence on AFM_1 content upon manufacturing of yoghurt or storage for 7 days at 7 °C (1993, El Deeb *et al.*, 1992 and Blanco *et al.*,). In contrast, (Bakirci, 2001, and Munksgaard *et al.*, 1987), detected variable increases of AFM_1content in yogurt related to the milk contamination.

Table (4):Content of AFM_1 in soft and hard white cheese after manufacturing.

Liquid milk with conc. of AFM_1 (µg/l or µg/Kg)	In soft cheese*			In hard cheese*		
	Range (Min-max) (µg/Kg)	Mean ±SE (µg/Kg)	Conc.(µg/l) Increase	Range (Min-max) (µg/Kg)	Mean ±SE (µg/Kg)	Conc.(µg/l) Increase
(0.5)	0.86-0.82	0.84±0.0071	1.68	1.18-1.23	1.21±0.0105	2.42
(0.25)	0.39-0.43	0.41±0.0071	1.64	0.57-0.62	0.59±0.0084	2.36
(0.05)	0.077-0.081	0.079±0.0007	1.58	0.111-0.116	0.114±0.0008	2.28

*** Contents of AFM_1after processing were obtained using 5 replicates, SE; Standard Error, figures are presented in the form of means ± SE.**

Table 5:Concentrations of AFM_1 (µg/l) and pH change after manufacturing of yoghurt at different intervals.

*** Contents of AFM_1after processing with means were obtained using 5 replicates; SD; Standard Deviation; SE, Standard Error.**

(Govaris *et al.*, 2002) reported that AFM_1 levels in all yoghurt samples showed a significant decrease from those initially present in milk. This decrease in AFM_1 was attributed to factors such as low pH, formation of organic acids or other fermentation by-products, or even to the proteins such as the caseins leading to formation of yoghurt coagulum. The change in casein structure during yoghurt production may affect the association of AFM_1 with this protein. (Rasic *et al.*, 1991) reported that the adsorption or occlusion of the toxin in the precipitate, causing $AxFM_1$ stability over storage of yogurt, is due to decrease in pH. (Govaris *et al.*, 2002) reported a high reduction (up to 97%) of AFM_1 in yogurt and acidified milk. For yogurt during refrigerated storage, it was reported that AFM_1 was rather more stable in the yoghurts with pH 4.6 than with pH 4.0. The percentage of loss of the initial amount of AFM_1 in milk was estimated to be 13 and 22% by the end of fermentation, and 16 and 34% by the end of storage for yoghurts with pH of 4.6 and 4.0, respectively.

Effect of some-detoxification methods on AFM_1 content

1. Effect of using ozone (O_3) gas on AFM_1 content.

Effect of detoxification using ozone gas on AFM_1 content is shown in Figures **1**&**2**. The ozone gas was applied for several chosen time intervals of 1, 2, 4, 6, 8, and 10 minutes on AFM_1-contaminated milk with two added concentrations of AFM_1 (0.5 and 0.25µg/l). Ozone treatment resulted in decrease or loss of AFM_1 content depending on the application time. The maximum effect was obtained with application of ozone for 10 minutes.

The percentages of minimization in the AFM_1 contents of 0.5 and 0.25 µg/l in milk samples were as follows: 24-23.2%; 35.2-34.4%; 48.8-46.4%; 66.4-64.8%; 82.4-81.2%; and 100-100%, corresponding to ozone treatment intervals of 1, 2, 4, 6, 8, and 10 min, respectively. After O_3 detoxification procedures, milk samples were further checked for screening and examining the presence of any content of AFM_1 using GC/MS (Figures 5-7). Nutritional factors and milk properties were also evaluated.

A statistical significant difference was found between the initial concentrations of AFM_1 and concentrations after treatment with ozone gas, showing a maximum effect at application of 10 minutes, at $P< 0.05$. Although, contents of AFM_1 were decreased or lost by ozone treatment, it was found that application of 10 minutes resulted in curd milk protein and change in its odor. The evidence of changing of milk properties with ozone application for 10 minutes, can state the conclusion that ozone gas is not suitable for milk detoxification.

Our results showed that treatment with ozone had a positive effect on elimination of AFM_1 but had a negative effect on properties of milk. Ozone, as a gas naturally found in the earth's atmosphere, has been used as a disinfectant agent for over hundred years. Ozone was first used to disinfect drinking water in the 19^{th} century and was approved in 1997, for use as a

disinfectant for food without leaving chemical residues. Ozone was reported to be an effective treatment for increasing shelf-life and decreasing fungal deterioration in the post harvesting treatment of fresh fruit such as table grapes (Sarig *et al.*, 1996).

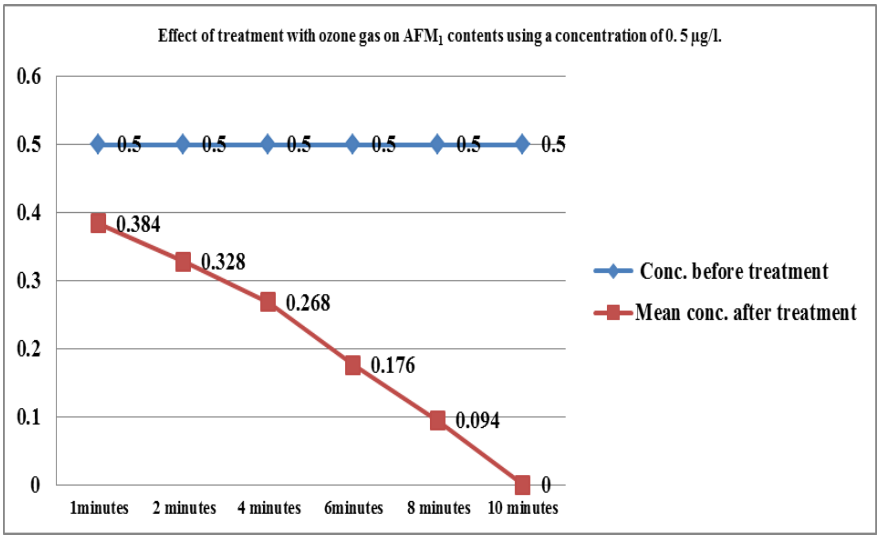

Figure 1: Effect of treatment of milk with ozone gas on AFM_1 contents using a concentration of 0. 5µg/l.

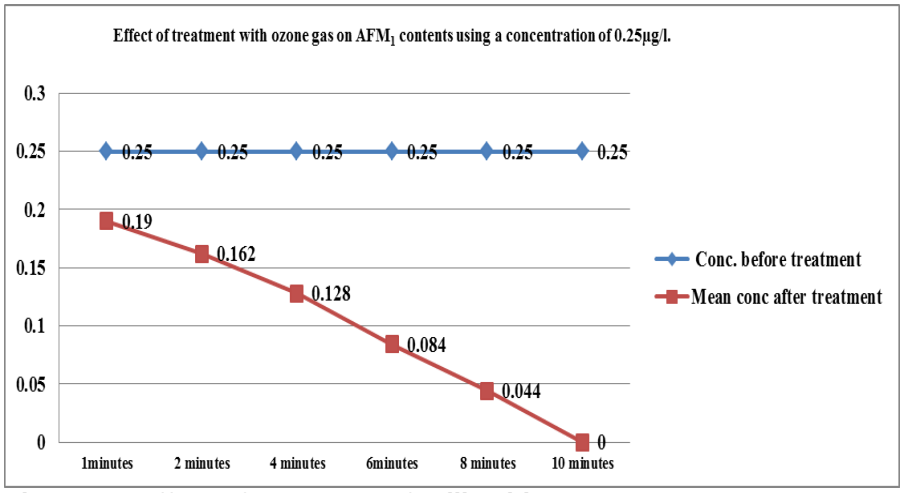

Figure 2 : Effect of treatment of milk with ozone gas on AFM_1 contents using a concentration of 0. 25µg/

2. Effect of treatment with gamma (γ) radiation on AFM_1

Effect of detoxification using gamma (γ) radiation on AFM_1 content is shown in Figures 3&4. The gamma (γ) radiation was applied in six doses of 0.5, 1.0, 1.5, 3.0, 5.0, and 10.0 kGy on AFM_1-contaminated milk with two added concentrations of 0.5 and 0.25 µg/l. Gamma (γ) radiation treatment resulted in decrease or loss of AFM_1 content depending on its applied dose. The maximum effect was obtained with application of gamma (γ) radiation at 10 kGy. The percentages of minimization in the AFM_1 contents of 0.5, 0.25 and 0.0 µg/l in milk samples were as follows: 3.2-5.6%; 7.6-9.6%; 12.4-17.6%; 26.4-28.8%; 50.8-54.40%; and 100-100% corresponding to exposure doses of 0.5, 1.0, 1.5, 3.0, 5.0, and 10.0 kGy, respectively. After gamma radiation detoxification procedures, milk samples were further checked for screening and examining the presence of any content of AFM_1 using GC/MS (Figures 5, 6 and 8). Nutritional factors and milk properties were also evaluated.

A statistical significant $p< 0.05$ difference was found between the initial concentrations of AFM_1 and concentrations after treatment with the six doses of gamma radiation, showing a maximum effect with 10.0 kGy,.

Contrary to ozone application, application of gamma (γ) radiation treatment resulted in a decrease or loss in contents of AFM_1 with no evidence of change in milk properties at any of the applied doses.

Our results are in agreement with those observed by several studies, as reported by Samarajeewa *et al.,* 1990 who indicated obvious elimination of aflatoxins, by almost 100%, in peanut meal by (γ) radiation. Also, Iqbal *et al.* (2012), reported 6% reduction of aflatoxin in hotpeppers after exposure to (γ) irradiation. Moreover, Farag *et al*., 1996 recorded significant reduction of the pure total aflatoxin and aflatoxin in grains after exposure to microwave irradiations. The effect of irradiation on the aflatoxin content of food and feed was previously shown by Aziz & Moussa, 2002, who reported that the degradation of AFB_1, observed in plum stored at refrigeration and irradiated at 3.5 kGy, decreased from 380-500 µg/ kg to 20 µg/ kg. The authors treated fruits with different gamma radiation doses and observed a progressive decrease in fungal count and mycotoxin levels (penicillic acid, patulin, cyclopiazonic acid, citrinin, ochratoxin-A and aflatoxin) at doses of 1.5 and 3.5 kGy. The authors reported also that no mycotoxins were detected in fruits treated with 5 kGy (Aziz & Moussa, 2002).

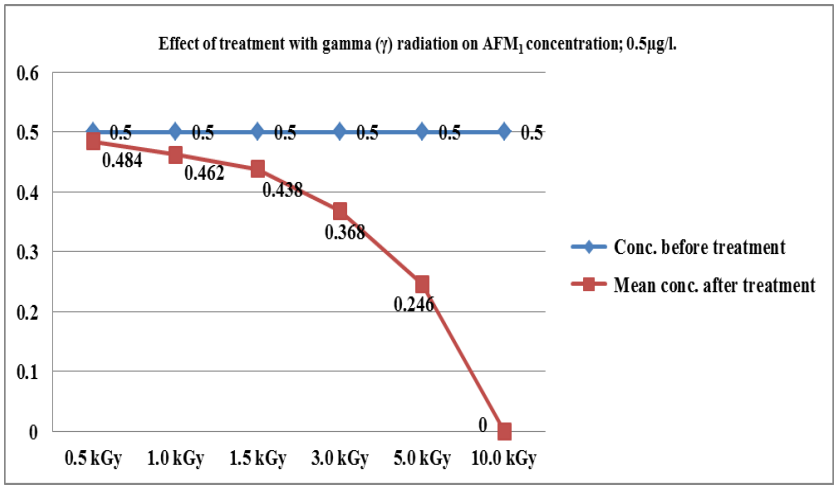

Figure 3: Effect of treatment of milk with gamma (γ) radiation on AFM_1 concentration; 0.5µg/l.

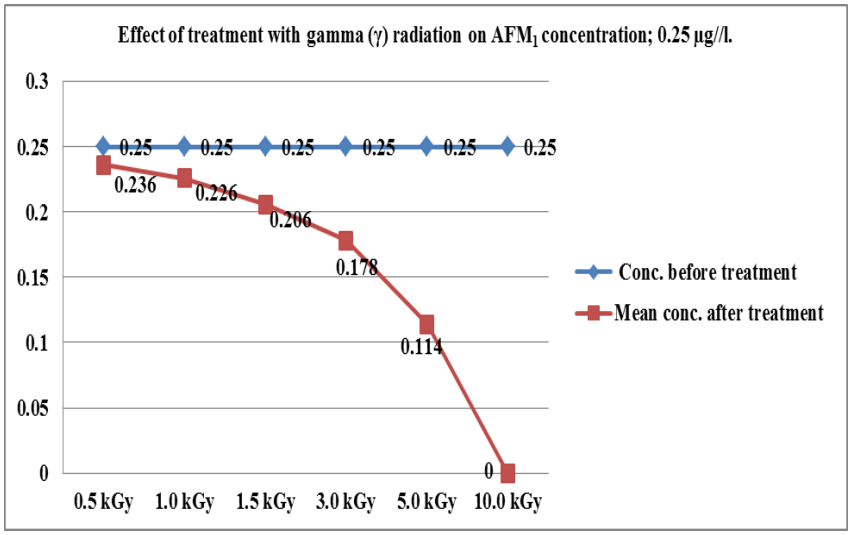

Figure 4: Effect of treatment of milk with gamma (γ) radiation on AFM_1 concentration; 0.25µg/l.

Figure 5: GC/MS for non-contaminated liquid milk sample, as a control. Integration peak list of non-contaminated liquid milk sample.

Figure 6: GC/MS for contaminated liquid milk sample, as a control. Integration peak list of contaminated liquid milk

Figure 7: GC/MS for contaminated liquid milk with AFM_1, exposed to ozone gas (O_3) in one dose of 200 mg/hr. for 10 minutes Integration peak list of contaminated liquid milk, exposed to ozone gas (O_3)

Figure 8: GC/MS for contaminated liquid milk sample, exposed to gamma (γ) radiation at high dose of 10 kGy Integration peak list of contaminated liquid milk, exposed to gamma (γ) radiation

3. Assessment of milk properties after ozone or (γ) radiation treatment.

Milk is a very complex food with over 100,000 different molecular species found. After detoxification treatment, milk properties and nutritional factors of the treated milk samples were examined and compared to those of non-treated, non-contaminated liquid milk samples. Milk properties were examined physically by odor and curd proteins. Nutritional factors such as; calories, fat, protein, carbohydrate, calcium, phosphor, vitamin B_1, vitamin B_2, were evaluated. Table **6** shows the nutritional factors of 100 ml milk samples after detoxification treatments which were compared to nutritional factors of non-treated and non-contaminated liquid milk samples**.** The contaminated samples, exposed to ozone gas (O_3) at one dose of 200 mg/hr for 10 minutes were tested for milk properties and mean nutritional factor. Exposure to ozone treatment resulted in curd milk protein and change in its odor, which gives evidence of a change in physical properties of milk and its mean nutritional factor. It was shown that the measured values of calories, fat, protein, carbohydrate, calcium, phosphor, vitamin B_1, and vitamin B_2 were 27.14kcal, 0.158gm, 2.042gm, 3.92gm, 109.08mg, 87.04mg, 0.021mg and 0.131mg, respectively.

The contaminated liquid milk samples exposed to gamma (γ) radiation at high dose of 10 kGy were also tested for milk properties and mean nutritional factor. Exposure to radiation treatment resulted in no change in milk's properties or nutritional factors. The mean measured values of calories, fat, protein, carbohydrate, calcium, phosphor, vitamin B_1, and vitamins B_2 were 31.02kcal, 0.20gm, 2.79gm, 4.52gm, 118.08mg, 92.96mg, 0.0406mg and 0.1738mg, respectively, and were almost similar to those values of non-treated, non-contaminated milk samples.

However, GC/MS examinations for milk samples treated with ozone gas or gamma radiation showed spectra identifying deletion of peaks of AFM_1, of a mass of 328kd, but treatments resulted in three other unknown peaks of known masses of 313, 297 and 257kd. These raises a question of what are these components; are these components safe to human health or not?. It seems that AFM_1 may be divided into three other unidentified compounds during detoxification treatments of milk. A future study, therefore, is needed to identify these compounds, verify their toxicity and their impact on human health.

Table 6:Nutritional factor for 100 ml milk samples after detoxification treatments compared to non-treated, non-contaminated liquid milk samples

*** Contents of AFM_1after treatments were obtained using 5 replicates SD; Standard Deviation; SE, Standard Error.**

In conclusion, the results of the underlying study indicated that AFM_1 could be found in dairy products manufactured from contaminated milk. Avoiding contamination seems to be the only practical way to ensure the safety of milk and milk products for human consumption. Despite of low incidence of AFM_1 in milk and other dairy products compared to other regions worldwide, more emphasis should be given to the determination of AFM_1 in milk and dairy products. Manufacturing of cheese from AFM_1-contaminated milk showed variable AFM_1 concentrations in soft and hard cheese higher than those in cheese milk, which may be attributed to association of AFM_1 with casein. Manufacturing of yoghurt resulted in a decrease in the contents of AFM_1, which may be attributed to factors such as low pH, formation of organic acids or other fermentation by-products. Treatment of contaminated milk by ozone gas resulted in a decrease or complete elimination of AFM_1in milk. It was found that 10 minutes of ozone treatment resulted in curd milk protein and change in its odor and properties; indicating that increasing treatment interval is not convenient for detoxification of milk or dairy products. However, treatment by gamma radiation at a dose of 10 kGy was found to be more suitable as a detoxification procedure than ozone treatment as there was no change in milk properties upon treatment.

Therefore, it is recommended in order to ensure safety consumption of milk and milk product daily intake, aflatoxin B_1 contaminated feeding for dairy cattle should be avoided. This seems to be the most practical way. Attention must be taken to consumption of guaranteed source to avoid severe contaminated with aflatoxin M_1 in milk and milk products. Using modern or new technologies for detoxification of milk should be further studied.

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رصد الأفلاتوكسين M_1 في بعض منتجات الالبان فى السوق المحلي للاسكندرية و محاولات لازالة السميه

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تم تحليل عدد ٢١٠ عينه من اللبن الخام، اللبن المبستر ، اللبن المعقم ، اللبن المركز ، لبن الرضع ، اللبن المجفف، اللبن الرائب، الزبادي وبعض انواع الجبن مثل الجبن القريش ، الجبن الابيض الدمياطى الطري ، الجبن الجاف، الجبن المطبوخ، والمثلجات اللبنية مثل الآيس كريم ، اللبنة ، الزبدة و مصل اللبن (الشرش) ، وتم تجميع العينات الاكثر استهلاكا وعشوائياً من الاسواق ومحلات السوبر ماركت في مدينة الإسكندرية بشمال جمهورية مصرالعربية خلال الفترة (٢٠١٢-٢٠١٣).وقد اظهرت تنتائج هذه الدراسة إلى وجود تلوث فى اللبن الخام ، اللبن المبستر ، اللبن المعقم ، اللبن المركز ، لبن الرضع ، اللبن المجفف ، اللبن الرائب ، الزبادي ، الجبن القريش ، الجبن الابيض الدمياطى الطري، الجبن الجاف ، الجبن المطبوخ، الآيس كريم ، اللبنة ، الزبدة و مصل اللبن (الشرش) ، وكانت النسبة المئوية للعينات هى (٣٧.٥، ٣٧.٣، ٥٤.٥، ٢٨.٥، ١٦.٦، ٣٣.٣، ٣٣.٣، ٢٦.٦ ،٣٣.٣، ٤٤.٤، ٢٥، ٢٩.٤، ٤٢.١، ٣٠.٧، ٢٥ و ٢٣.٠٨) على التوالي.

جميع العينات الإيجابية للأفلاتوكسينM_1 من اللبن الخام، اللبن المبستر ، اللبن المعقم ، اللبن المركز ، لبن الرضع ، اللبن المجفف، اللبن الرائب، الزبادي ، الجبن القريش ، الجبن الابيض الطري ، الجبن الجاف، الجبن المطبوخ ، الآيس كريم ، اللبنة، الزبدة ومصل اللبن (الشرش) ، لم تتجاوز الحدود المسموح بها للولايات المتحدة (٠.٥ ميكروجرام/لتر أو ميكروجرام/كيلوجرام). بعض العينات الإيجابية من اللبن الخام و اللبن المبستر و اللبن المجفف كانت تتجاوز الحدود المسموح بها للدول الأوروبية (٠.٠٥ ميكروجرام/لتر أو ميكروجرام/كيلوجرام)، وتتجاوز الحدود المصرية المسموح بها (٠.٠٥ ميكروجرام/لتر أو ميكروجرام/كيلوجرام) ، بينما جميع العينات بنسبة ٣%، ٢% و ٣% على التوالي.

نتائج عملية تصنيع الجبن الابيض الدمياطي الى ان تركيز الافلاتوكسين M_1 في الجبن الطري و الجبن الجاف ١.٧٧أضعاف تركيزه فى اللبن المصنع منه الجبن وهذا قد يرجع الى ارتباط الافلاتوكسين M_1 مع (الكازين) بروتين اللبن (الافلاتوكسين M_1 قابل للذوبان بشكل رئيسي في المرحلة المائية من اللبن أوالادمصاص على سطح جزيئات الكازين) .

أشارت نتائج عملية تصنيع الزبادى الى انخفاض نسبة تركيز الافلاتوكسين M_1 وهذا الانخفاض قد يرجع إلى عدة عوامل مثل انخفاض الرقم الهيدروجيني ، وانتاج الأحماض العضوية أو التخمير وغيرها من العوامل .

اظهرت نتائج المعاملات للتخلص من الافلاتوكسينM_1 باستخدام غاز الأوزون ،وذللك باستخدام لبن تم تلويثة بتركيزين من الافلاتوكسينM_1 وهى (٠.٥ و ٠.٢٥ ميكروجرام/لتر) والتي كانت جرعة التعرض ٢٠٠ ملجم / ساعة ، فانخفض متوسط التركيزات للافلاتوكسينM_1 انخفضت بنسبة ٢٤-٢٣.٢ ٪ ، ٣٥.٢-٣٤.٤ ٪ ، ٤٨.٨- ٤٦.٤٪ ،٦٦،٤٤-٦٤،٨ ٪ ، ٨٢.٤-٨١.٢ ٪ و ١٠٠-١٠٠٪ لمدة ١ ، ٢ ، ٤ ، ٦ ، ٨ و ١٠ دقيقة ،على التوالي .

اظهرت نتائج المعاملات للتخلص من الافلاتوكسينM_1 باستخدام أشعة جاما (γ) ، وذللك باستخدام لبن تم تلويثة بتركيزين من الافلاتوكسينM_1 وهى (٠.٥ و ٠.٢٥ ميكروجرام/لتر) والتي تم تعرضها لعدد ٦ جرعات لأشعة جاما (γ) ، هى (٠.٥ كيلو جراى ، ١.٠ كيلو جراى ، ١.٥ كيلو جراى ، ٣.٠ كيلو جراى ، ٥.٠ كيلو جراى ، ١٠.٠ كيلو جراى ان متوسط تركيزات للافلاتوكسينM_1 انخفضت بنسبة ٣،٢-٥،٦ ٪ ، ٧.٦-٩.٦ ٪ ، ١٢.٤-١٧.٦ ٪ ، ٢٦.٤-٢٨.٨٪ ٥٠.٨-٥٤.٤٪ و ١٠٠-١٠٠٪ ، على التوالي.

ادى لتخثر بروتين اللبن و تغيير رائحته وقد أعطى هذا دليل على تغيير خواص اللبن ، لذلك يمكننا القول ان غازالأوزون غير مناسب للمعاملة مع الالبان ، على عكس المعاملة بواسطة أشعة جاما كانت مناسبة واكثر امانا لخواص اللبن وكذلك القيمة الغذائية والاستهلاك البشرى.

قام بتحكيم البحث

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