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Stability of Aflatoxin M_1 During Manufacture and Storage of Yoghurt, Yoghurt-Cheese and Acidified Milk

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Abstract:

Yoghurt, yoghurt-cheese and acidified milk were prepared from pasteurized milk half cream, naturally contaminated with aflatoxin M_1 and then were stored at 4° for two weeks. Reduction in levels of aflatoxin M_1 during manufacture and storage has been investigated. These dairy products were analyzed for aflatoxin M_1 content at two day intervals, for two weeks. Levels of aflatoxin M_1 in these dairy products were high, and then began to decrease during storage period at 4°. In yoghurt, aflatoxin M_1 recoveries 40.9% (pH values ranged from 5.10 to 4.70 and titratable acidity ranged from 0.23% to 0.31%) during storage period. Whilst the results from yoghurt-cheese gave aflatoxin M_1 recoveries 70.6%, where pH values during storage period ranged from 5.15 to 4.65 and titratable acidity ranged from 0.39% to 0.35%. When acidified milk was used the percentage recoveries of toxin 34.2% after two weeks, where pH values ranged from 2.95 to 2.85 and titratable acidity ranged from 0.23% to 0.22% during storage period.

Introduction:

Aflatoxin M_1 was identified as a monohydroxylated metabolite of AFB (Asao et al., 1963 and Holzapfel et al., 1966), and was called aflatoxin M_1 (AFM).

Several studies (Allcroft and Roberts, 1968, and Masri et al., 1969) have been done to determine the relationship between ingestion of aflatoxin and excretion of aflatoxin M_1 in milk, when significant quantities of AFB_1 are consumed, AFM_1 appears in milk at least 12 h postfeeding. Ground peanuts and extracted ground nut meal from oil refineries are mainly used for mixing with milk cattle feed. Approximately 1% or even more of the original aflatoxin B_1 content in the diet is excreted as aflatoxin M_1 in the milk (Kiermeier, 1977 a and 1978). Conversion of the major contaminating aflatoxins into those of the M series is by the animal's hepatic microsomal mixed function oxidase system (Lynch, 1979). Aflatoxin M_1 has been found in commercial samples of cheeses (Kiermeier et al., 1977 b, and Polzhofer, 1979). In 1979 a survey

of manufactured dairy products produced in the USA (992 samples of nonfat dry milk, vanilla ice cream, yoghurt, cheddar cheese and cottage cheese) for AFM_1 contamination, one sample, a cottage cheese, had detectable aflatoxin equivalent to 0.08 ng/ml (ppb) in the milk from which the product was made (Stoloff, et al., 1979).

The purpose of this work is to investigate the fate of aflatoxin M_1 in yoghurt, yoghurt-cheese and in acidified milk during manufacture and storage.

Materials and Methods:

Juhayna [milk half cream; Ultra Heat treated (UHT) cow milk; 1.5% fat-8.5% solids-not-fat (SNF)] was purchased from an Egyptian local market.

The aflatoxin M_1 was obtained from Sigma Chemical Co., St. Louis. MO, U.S.A. It was used as reference standard.

Preparation of yoghurt, yoghurt-cheese and acidified milk:

Yoghurt:

using pasteurized milk half cream (40°), an ethanol solution of aflatoxin M_1 was added to obtain concentration of 10 ppm (0.01 mg/ml), mixed well and inoculated with 3% of yoghurt culture, followed by incubation at 42° for 4.5 h until coagulation (pH value was 5.10). One sample was then analyzed for aflatoxin M_1 (zero time). Yoghurt samples were placed in the refrigerator at 4°. At two day intervals, for two weeks, the AFM_1 content was extracted and determined in triplicate samples (50 g/sample). Toxin-free sample was used also as control.

Yoghurt-cheese:

The same preparation as mentioned in yoghurt was applied to yoghurt mixed with 7.5 g/L NaCl, followed by incubation at 42° for 5 h and 20 min. until coagulation (pH value was 5.15). Then yoghurt- cheese was put into cheese cloth bags to allow whey drainage. Yoghurt-cheese was filled into 1.050 kg plastic containers and kept in the refrigerator at 4° for analysis. The analysis was done as described before in triplicate samples (50 g/sample). Toxin free sample was used also as control.

Acidified milk:

Pasteurized milk half cream was acidified to 2.95 by the addition of a 20% water solution of acetic acid, an ethanol solution of AFM_1 was added to obtain concentration

of 10 ppm. One sample was analyzed for AFM_1 (zero time). Samples were held in the refrigerator at 4° and then analyzed for AFM_1 content as mentioned before. Toxin-free samples were used also as controls.

Determination of aflatoxin M_1:

Aflatoxin M_1 was extracted from products and then analyzed using the method of Stubblefield and Shannon (1974). The chloroform extract was filtered through anhydrous sodium sulphate , evaporated to dryness under nitrogen and stored at 3.5° before being redissolved in 100 µl chloroform before use. Thin layer chromatography pre-coated plates (sigma aluminum-backed silica gel layer 250 µm on polyester, without fluorescent indicator, activated at 110° for 30 min.) with yoghurt, yoghurt-cheese and acidified milk samples were developed in ether-methanol-water (95:4:1, v/v/v). Fluorescence of AFM_1 spots were measured with a fluorodensitometer CD60 at an excitation wavelength of 365 nm and emission wavelength of 443 nm.

The amount of aflatoxin M_1 extraction given is the mean of three sample replicates on one TLC plate, and each spot was scanned twice.

Total titratable acidity in yoghurt, yoghurt-cheese and acidified milk were determined according to the method of Kent-Jones and Amos, (1967) and Pearson, (1981). Total acidity (%) was calculated as lactic acid. Hydrogen-ion concentration (pH) was measurement using a pH meter (Orion Research, model 201 / digital pH meter) as indicated by the official A.A.C.C. (1970) method.

Statistical analysis was done by the completely randomized design in factorial arrangement (F-test). Least significant difference (L.S.D.) was used for comparing treatment means (Snedecor and Cochran, 1980).

Results and Discussion:

Table (1) and Fig. (1) give the aflatoxin M_1 recovery (%) of yoghurt, yoghurt-cheese and acidified-milk during storage period (two weeks). Most of the AFM_1 was recovered from yoghurt-cheese 70.6% ($p < 0.01$-Table 3), pH values during refrigerator storage ranged from 5.15 to 4.65 and titratable acidity ranged from 0.39% to 0.35%, (Table 2) whereas yoghurt contained only 40.9% of AFM_1 ($p< 0.01$-Table 3), values of pH ranged from 5.10 to 4.70 and titratable acidity ranged from 0.23% to 0.31%. On the other hand the greatest decrease in AFM_1 added at a concentration of 10 ppm could be detected in milk acidified with acetic acid 34.2% ($p < 0.01$-Table 3), where the pH

values ranged from 2.95 to 2.85 and titratable acidity ranged from 0.23 to 0.22% during refrigerator storage, Table (2). These results agree with those of others (Allcroft and Carnaghan, 1963; Mckinney et al., 1973; Kiermeier and Buchner, 1977 c) where they demonstrated an increased concentration of AFM_1 in the curd over that in milk. Whereas Jeremija et al., 1991, studied the decrease of AFB_1 in yoghurt and acidified milk, and found that, a decrease of 97% in yoghurt samples with 6000 μg/kg AFB_1, and 90% in those with 1400 μg/kg, and a greatest decrease of 90 % in milk acidified samples with 1000 μg/kg.

The results (Table 1) show an increase in recovery of AFM_1 after manufacture of dairy products. However, the maximum amount of AFM_1 for yoghurt, yoghurt-cheese, whey and acidified milk were 83.4%, 87.9%, 88.5% and 85.6% respectively ($p < 0.01$-Table 3). These results are in agreement with those of Polzhofer (1977), who found a 9% decrease in toxin after manufacture of process cheese. A studies have been concerned with the behaviour of AFM_1 during the manufacture of butter from contaminated cream (Grant and Carison, 1971; Kiermeter and Mashaley, 1977 d and Stubblefield and Shannon, 1974). Two of these studies that monitored partitioning of AFM_1 between butter and buttermilk used artificially contaminated cream (Grant and Carison, 1971; Stubblefield and Shannon, 1974). One study monitored the partitioning of AFM_1 when both fresh and cultured cream were churned into butter (Kiermeter and Mashaley, 1977 d). In all these studies, most of the AFM_1 initially in the cream was recovered from buttermilk or rinse waters.

On storage, the AFM_1 contents of dairy products were varied. However, the aging process could result in changes, which allow recovery of more or less toxin. The primary changes which occur during the aging of dairy products may be proteolysis and lipolysis besides the changes in values of pH. Also, the acidity increases due to the action of micro-organisms, and a sour taste is perceptible when this reaches about 0.3%-0.4%, Tables 1 and 2. These results conflict with those of Robert and Elmer, 1982 who found that the AFM_1 content of cheese spread appeared to increase, and then return to near original levels during storage at 7°. Whilst Dana and Elmer, 1983, repotted that the AFM_1 remained in bakers cheese through 1 month of refrigerated storage and 2 months of frozen storage.

Also in 1983, Dana and Elmer studied the stability of AFM_1 during manufacture and storage of a butter-like spread, non-fat dried milk and dried buttermilk. They found that, during refrigerated storage for 1 month and frozen storage for up to 2 months, the

AFM_1 content was variable but toxin remained in the butter-like spread.

Mohran et al., 1984, studied the effect of AFB_1 on the proteolytic activity of some lactic acid bacteria, their data revealed that aflatoxin in milk can affect the lactic acid bacteria which are used in the manufacture of dairy products. Such effect depends on toxin concentration and the species of lactic acid bacteria.

In a study on aflatoxin M_1 in human breast milk, some investigators have examined human exposures to aflatoxins from the diet (Groopman et al., 1991). Methodological constraints have inhibited extensive investigations to assess maternal to child exposure from breast milk to a major carcinogenic metabolite of aflatoxin B_1 (AFB_1), aflatoxin M_1 (AFM_1). AFM_1 is a hydroxylated derivative formed in humans by cytochrome P 450 1A2 (Faletto et al., 1988) and is carcinogenic for both the liver and colon in rats (Cullen, et al., 1987). Thus the formation of this compound is not a detoxification pathway. This metabolite has been found to be a major excretion product in the milk of lactating animals and women exposed to dietary AFB_1 (Wild et al., 1987 and Scott, 1989). In a study on AFM_1 in human breast milk from the Gambia, West Africa, Audrey et al., 1992, reported that, the estimates of the percentage of aflatoxin in the diet excreted as AFM_1 in milk ranged from 0.09% to 0.43%.

The results also show the coagulation time for experimental and control samples of yoghurt milk was approximately the same (4.5 h), pH values and titratable acidity were 5.10 (0.23%) and 5.15 (0.25%) for experimental and control respectively. Whilst the coagulation time for yoghurt-cheese and control samples was 5 h and 20 min., values of pH and titratable acidity were 5.15 (0.39%) and 5.10 (0.40%) for experimental and control respectively Table (2).Yoghurt bacteria in yoghurt and yoghurt-cheese with AFM_1 had longer chains in experimental samples than in the controls. Other research workers observed morphological changes of both streptococci and lactobacilli in yoghurt samples containing much higher concentrations of AFM_1 (Sutic and Banina, 1979). Banina and Topisirovic 1990, reported that AFB_1 has shown a toxic effect on streptococcus lactic and inhibited its growth. But inhibition was partial at lower and complete at higher concentration of AFB_1. Cells grown in the presence of AFB_1 were filamentous and formed much longer chains than when they grew without AFB_1.

Conclusion:

When milk contaminated with AFM_1 is used to produce dairy products. The AFM_1 in the milk is transmitted to manufactured dairy products. Results of this research show that the AFM_1 tended to concentrate in fractions containing casein. It is therefore possible for AFM_1 to reach consumers if milk contaminated with the toxin is used to manufacture such products.

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Table (1): Aflatoxin M_1 recoveries in dairy products produced from artificially contaminated milk.

Dairy product	Aflatoxin M_1 recovery %*						
	1st day (0-time)	3rd day	5th day	7th day	9th day	11th day	13th day
Yoghurt	83.4	67.8	65.0	59.5	58.2	53.1	40.9
Yoghurt-cheese	87.9	83.5	83.4	82.3	79.2	78.7	70.6
Whey	88.5	—	—	—	—	—	—
Acidified-milk	85.6	78.2	67.6	63.3	61.8	56.3	34.2

* Percentages were calculated on M_1 added to original milk.

Table (2): The effect of storage (14 days) on pH values and titratable acidity for yoghurt, yoghurt-cheese and acidified milk at refrigerator temperature 4°.

Days	Yoghurt				Yoghurt-cheese				Acidified milk			
	pH		Titratable* acidity		pH		Titratable acidity		pH		Titratable acidity	
	sample	Control	sample	Control	sample	Control	sample	Control	sample	Control	sample	Control
1	5.10	5.15	0.23	0.25	5.15	5.10	0.39	0.40	2.95	2.85	0.23	0.22
3	5.10	5.15	0.23	0.25	5.00	4.95	0.38	0.39	2.95	2.85	0.23	0.22
5	4.85	4.85	0.39	0.39	4.75	4.80	0.36	0.37	2.85	2.85	0.22	0.22
7	4.85	4.85	0.39	0.39	4.75	4.80	0.36	0.37	2.85	2.85	0.22	0.22
9	4.85	4.85	0.39	0.39	4.70	4.80	0.35	0.37	2.85	2.85	0.22	0.22
11	4.75	4.85	0.32	0.39	4.70	4.80	0.35	0.37	2.85	2.85	0.22	0.22
13	4.70	4.75	0.31	0.40	4.65	4.75	0.35	0.37	2.85	2.85	0.22	0.22

* Calculated as lactic acid (%).

Table (3): Analysis of variance for the concentration of aflatoxin M_1 remained after storage.

Sources	DF	F-test		Coefficient of variation
		AFM_1	L.S.D.	
A	2	279.177**	0.189	3.595%
B	6	131.617**	0.289	
AB interaction	12	14.950**	0.501	
Error	21	--		
Total	41	--		

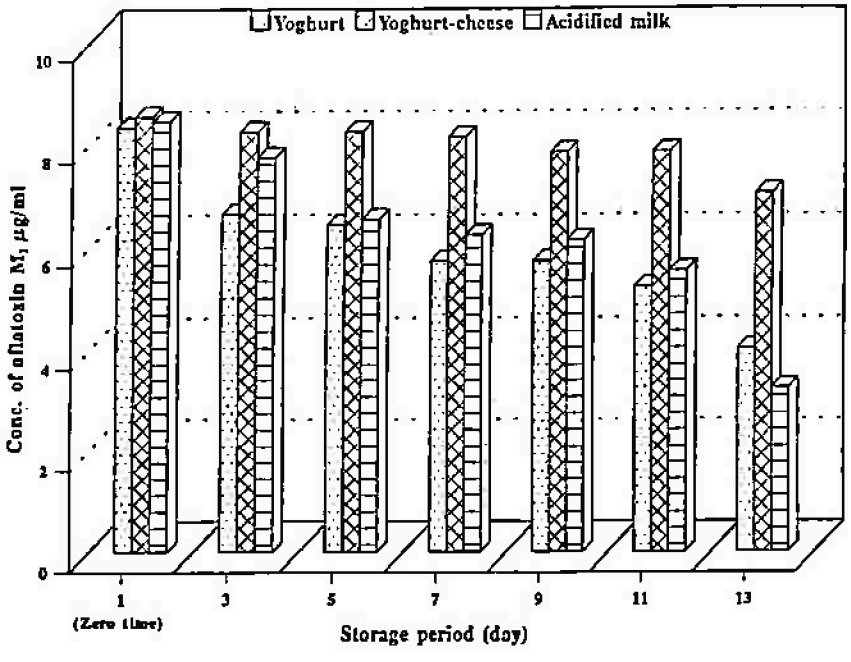
A = Treatments (yoghurt, yoghurt-cheese and acidified milk).

B = Storage days.

L.S.D.= Least Significant Difference.

** = $p < 0.01$.

Fig (1): Aflatoxin M_1 content of yoghurt, yoghurt-cheese and acidified milk during storage at 4°C



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