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FATE OF AFLATOXIN M_1 DURING MANUFACTURE AND STORAGE OF EGYPTIAN KARISH SOFT CHEESE

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ABSTRACT

Aflatoxin M1 (AFM1) is an important mycotoxin frequently found in milk and dairy products. Egyptian karish cheese from raw cow's milk artificially contaminated with AFM1 at level of 100 ng/L (ppt) was fermented to reach pH 3.8 ± 0.04. The samples were stored at 4°C for up to 3 weeks. Analysis of AFM1 in karish cheese samples was carried out using immunoaffinity column coupled to indirect competitive enzyme-linked immunosorbent assay (ELISA) method. AFM1 in cheese showed a significant increase ($P< 0.01$) compared with those initially added to raw milk. The level of AFM1 in cheese samples were 3.5 ±0.07 fold. During the refrigerated storage AFM1 was rather more stable, the percentage losses were zero, 3.75±0.05, 6.25±0.16 and 13.75 ±0.24 by the end of storage with pH 4.1±0.08, 3.1±0.01, 2.5±0.03 and 2.2±0.01, respectively. Changes in AFM1 content of cheese samples were statistically insignificant ($P>0.01$) during 3 weeks storage. The results indicate that the necessary precaution has to be taken to minimize the AFM1 contamination in milk.

Keywords: Soft Karish Cheese, Aflatoxin M1, Stability During Storage

INTRODUCTION

Karish cheese is one of the most popular fresh skim milk soft cheese in Egypt. The increasing demand by Egyptian consumers is mainly attributed to its high protein content and low price **[1]**. Karish cheese is traditionally made from raw skim cow or buffalo's milk which is milked directly into special earthenware pots known as (shalia) and kept undisturbed in a suitable place to allow the fat to rise to the surface forming a layer. Then the cream layer is removed and the curd is poured onto a mat which is tied and hung with its contents to allow the drainage of the whey. This process of

squeezing takes two or three days until the desired texture of the cheese is obtained. Finally, the cheese is cut into suitable pieces and salted cheese is left for a few hours in the mat till whey no longer drains out, then it is ready to be consumed as fresh soft cheese **[2]**. Aflatoxins, a group of highly toxic secondary fungal metabolites produced by some *Aspergillus* species, are found in a wide variety of foods and feeds around the world. Aflatoxin B_1 (AFB_1) is the most frequent form present in contaminated foods and feeds **[3].** Ingestion of aflatoxin B_1 contaminated feed by lactating animals lead to excretion of aflatoxin M_1 (AFM_1), 4 hydroxylated metabolite of AFB_1, in milk **[4]**. Although children and elderly are the major milk and dairy products consumers, the quality of milk products has a strong influence on the health of people in various age brackets. AFM_1 is known to be hepatotoxic and carcinogenic. The World Health Organisation changed its classification from group 2 to group 1 **[5]**.

Because of the increased demand for skim milk karish cheese by consumers, the goal of the research, reported in this article, was to study the behavior of AFM_1 during production and refrigerated storage for three weeks.

MATERIALS AND METHODS

Manufacture of Skim Milk Karish Cheese

Raw skim cow,s milk (10 L) purchased from Cairo market, Egypt (free from AFM_1) were spiked with standard aflatoxin M_1 (Sigma Chemical Co., Irvine, UK) according to **[6]** at the level of 100 ng/L (ppt). The samples were warmed to 42°C to be mixed with 1% sodium chloride and commercial starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (V1-Hansen, Denmark), was added at the level of 1.5% (w/w), leaving it for 15 min, then rennet (Sigma–Aldrich, 5876) and calcium chloride (Sigma–Aldrich, 5080), 0.015% were added. Following mixing, the samples were dispensed into 40 sterile polyethylene bags and incubated at 45ºC for about 7 h until coagulation occurred. Once the milk was coagulated, the coagulum was cut into 2×2 cm^3 pieces and the whey was drained off. The curd then was placed in perforated moulds in the refrigerator at 4ºC and examined for AFM1 after 7, 10, 14 and 21 d, respectively. The pH value was measured using pH meter (Orion, digital pH) during the storage period.

Analysis of AFM1

Karish cheese samples (50 g) were mixed with 75 ml chloroform, 1 ml saturated sodium chloride solution and 5 g Celite hyflosupercell for 45 min with continuous stirring. The mixture was filtered through filter paper 110 mm (Schleicher and Schull, Germany). The chloroform extract was

poured into 250 ml flask, evaporated to dryness in water bath (Edelstahl, UK) at 30ºC. One ml methanol, 30 ml distilled water and 50 ml hexane, were added to the residue. The mixture was transferred to a separating funnel and washed 2 times with 10 ml distilled water with periodical shaking for 15 s. The lower layer was collected and passed through the immunoaffinity column (Aflaprep M R.Biopharm Rhone Ltd) for clean up according to **[7]**. Determination of aflatoxin M1 using indirect enzyme-linked immunosorbent assay method (ELISA) according to **[8].** The detection limit was 20 ng/kg. Recoveries of AFM1 were 95%. Analytical results were corrected for recovery. All data were subjected to analysis of variance (ANOVA), whereas differences between means were tested for significance by Ducans multiple range test in the general linear model of SPSS statistical package (SPSS, 10.05, SPSS Ltd, Working, UK). Differences between means were considered as significant at $P<0.01$.

RESULTS AND DISCUSSION

The mean concentration of AFM1 in skim milk karish cheese samples was 350 ± 0.09 µg/kg. The enriched factor was 3.5 ± 0.07 fold **(Table 1).**

Nearly similar findings were reported by **[4, 9-11]** who observed variable increases of aflatoxin M1 content in cheese related to that in the milk. The higher concentration of aflatoxin M1 in cheese may be due to the favorableness of AFM1 to protein specially ß-casein **[12, 13].** The increased toxin content in karish cheese possibly results from a more recovery of aflatoxin M1 in the manufactured cheese than the original raw milk **[14, 15].** In contrast **[16, 17]** reported that, there was no influence of cheese manufacture on aflatoxin M1 content. The contradictory results on the stability of AFM1 during manufacture of cheese might be due to several factors as the different pH, various concentration of AFM1 in milk, use of different extraction techniques and methods of determination **[18, 19]**.

The storage of cheese samples at 4°C for 7, 10, 14 and 21 d gave mean percentage losses of zero, 3.75 ± 0.05, 6.25 ± 0.16 and 13.75 ± 0.24 at mean pH values 3.4 ± 0.08, 3.1 ± 0.01, 2.5 ± 0.03 and 2.2 ± 0.01, respectively **(Table 2).**

The level of AFM1 in cheese samples did not significantly change ($P> 0.01$) during the storage period. The relatively stable level of AFM1 in karish cheese samples during storage might be attributed to the affinity of AFM1 for caseins which may cause concentration effect during milk process as well as the rather stable pH values **[12]**. Nearly similar findings were recorded by **[11, 17]** who found that AFM1 was stable during storage for 7 d at 7°C. In contrast **[18, 20]** reported a high reduction of AFM1

during 13 d of refrigerated storage at 4ºC. The decrease of aflatoxin M1 during cold storage may be due to the dissociation of milk casein particularly ß-casein and its subsequent solubility in milk serum **[21, 22]**. Increasing acidity by prolonged storage was not responsible for aflatoxin inhibition. This agreed with **[12, 23, 24]** who found that 85% lactic acid did not affect aflatoxins.

Table 1: Stability of Aflatoxin M1 During Manufacture of Karish Cheese

CONTAMINATED MILK (ng/L)	MEAN AFM1 IN MANUFACTURED KARISH CHEESE (ng/kg)	MEAN ENRICHED FACTOR	MEAN pH VALUE
100[a]	350*[b] ± 0.09	3.5± 0.07	3.8±0.04

***Mean Value ± SD, Superscript (a–b) Differ Significantly (P<0.01)**

Table 2: Stability of Aflatoxin M1 (350 ng/ kg) During Refrigerated Storage of Karish Cheese

TIME/D	MEAN PERCENTAGE LOSSES (%)	MEAN pH
0	0[a]	3.8 ± 0.04
7	0[b]	3.4 ± 0.08
10	3.75 ± 0.05[b]	3.1 ± 0.01
14	6.25 ± 0.16[b]	2.5± 0.03
21	13.75 ± 0.24[b]	2.2 ± 0.01

*** Data in the Same Column with a Superscript (a - b) do not Differ Significantly (P>0.01)**

CONCLUSIONS

Briefly, the results of this study indicated that AFM1 could be available in dairy products made from contaminated milk. Therefore, avoiding contamination appears to be the only practical way to ensure the safety of milk products for human consumption.

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