Detection of Aflatoxin M1 in Pasteurized Canned Milk and Using of UV Radiation for Detoxification

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Abstract--The current study was designed to investigate the presence of aflatoxin M1 in 25 samples of pasteurized canned milk which collected randomly from some Iraqi local markets using ELISA technique. Aflatoxin M1 was present in 21 samples, the concentration of aflatoxin M1 ranged from (0.25-50 ppb).
UV radiation (365nm wave length) was used for detoxification of aflatoxin M1 (sample with highest concentration /50 ppb of aflatoxin M1 in two different volumes ((25 & 50 ml)) for two different time (15 & 30 min) and 30, 60, 90 cm distance between lamp and milk layer were used for this purpose). Results showed that distance between lamp and milk layer was the most effective parameter in reduction of aflatoxin M1, and whenever the distance increase the effect also increase.

Keywords- Aflatoxin M1, detection, Milk, UV radiation, Detoxification

I. INTRODUCTION

Mycotoxins are secondary metabolites produced by various fungi [1]. Aflatoxins are fungal metabolites produced by three species of *Aspergillus*, namely *A. flavus*, *A. parasiticus* and *A. nomius*. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. One of the mycotoxins, aflatoxin M1 (AFM1) is the hydroxylated metabolite of aflatoxin B1 (AFB1) and can be found in milk and subsequently in other dairy products when lactating animals are fed with contaminated feedstuffs [2].

About 1-2% of aflatoxin B1 in animals feed is transformed to AFM1 in milk, it may vary from animal to animal. 12-24 h after the first aflatoxin B1 ingestion, the toxin can be detected in the milk [3].

The occurrence of aflatoxin M1 in milk is transitory in nature and reaches maximum within two days after the intake of the contaminated commodity [4]. Aflatoxins represent a serious risk for animal and human health, especially for children, who are the major milk consumers [5].The hepatotoxic, genotoxic, carcinogenic, teratogenic, immunosuppressive and antinutritional effects of aflatoxins are well documented [6].

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Aflatoxins are considered to be human liver carcinogens, Aflatoxin B1 being the most potent. Aflatoxin M1 has a potency approximately one order of magnitude lower than that of aflatoxin B1 [7]. The presence of aflatoxin M1 in milk and dairy products can be a potential threat to the health of consumers [8]. Exposure to aflatoxin M1 through milk products is a serious problem for public health. Several countries have established regulatory limits for aflatoxin M1 in raw milk and milk products, which vary from country to country [9].

At present, aflatoxin presence in feed, milk and dairy products can be systematically controlled in Europe and other developed countries [10]. The European Community has set the maximum permitted level for aflatoxin M1 in raw milk and heat-treated milk at 0.05 μg L-1 [11]. Various physical, chemical and biological agents have been used to detoxify aflatoxins from food and feed materials [12]. However, no universally applicable, effective and practical methods are currently available [13]. Ultraviolet (UV) energy can be used effectively to inactivate aflatoxin M 1 in milk [14]. UV irradiation has been discovered for many years as an effective physical method to destroy aflatoxins for its photosensitive property [15] .Rate of degradation was a function of the film thickness and depth of penetration of the rays when operating conditions were held constant [16]. Because aflatoxins are photosensitive [15], our aim was to study the detection of aflatoxin M1 and detoxification of it in milk using ultraviolet (UV) energy at different distance between UV source and milk and different volumes of milk at different times.

II. MATERIAL AND METHODS

A. Samples collection:

Twenty five sample of liquid canned milk were randomly collected from some Iraqi local markets which Listed in the following table-1:

Detection of aflatoxin M1 in milk samples using Enzyme Linked Immune Sorbent Assay (ELISA) technique:

Detection of aflatoxin M1 by ELISA technique was performed using ELISA kit which supplied from Shenzhen Lvshiyuan Biotechnology company.

Determination of aflatoxin M1 concentration:

The concentration % of aflatoxin M1 in test samples was calculated using aflatoxin M1 standard curve according to the following equation:

Percentage of absorbance value = B / B0 × 100 %

B = the average OD value of the sample or the standard solution.

B0 = the average OD value of the 0 ng/ml standard solution.

Irradiation of samples

TABLE I
DIFFERENT MILK SAMPLES WITH THEIR ORIGIN

Milk brand	Origin
KDD full cream	Kuwait
KDD half cream	Kuwait
KDD light cream	Kuwait
KDD skimmed	Kuwait
Süt aysah	Turkey
Alsafi low fat	Saudi Arabia
Almarai full cream	Saudi Arabia
Almarai half cream	Saudi Arabia
Almarai light cream	Saudi Arabia
Almarai skimmed	Saudi Arabia
Nada full cream	Saudi Arabia
Nada half cream	Saudi Arabia
Nada light cream	Saudi Arabia
Nadec full cream	Saudi Arabia
Nadec half cream	Saudi Arabia
Nadec light cream	Saudi Arabia
Pinar	Turkey
Milky way	Egypt
Mars	Egypt
Juda	Kuwait
AL Rai	Saudi Arabia
Hammoudeh Banana flavor	Jordan
Safio strawberry flavor	Saudi Arabia
Safio Banana flavor	Saudi Arabia
YAG GO vanilla flavor	France

Ultraviolet source

The source of UV energy was a UV lamp (VISION science Co., Ltd, Korea), the main wavelength of the Lamp was 365 nm. The lamp was elevated to give a distance between lamp and milk layer of (30, 60, 90 cm) respectively.

UV Treatment:

Different volumes of contaminated milk sample (sample with highest concentration /50 ppb) (25 mL & 50 mL) was exposed to UV radiation (365 nm) for (15 & 30 min) and the residual aflatoxin M 1 content was measured at the end of each exposure.

Statistical analysis

All analytical determinations were performed at least in triplicate using SPSS program v 11.5. Values of different parameters were expressed as the mean ± standard error using student T-test. A defferences of $P \leq 0.05$ was considered statistically significant.

III. Results And Discussion:

Detection of aflatoxin M1 in milk samples using Enzyme Linked Immune Sorbent Assay (ELISA) technique:

Our results showed that twenty one samples of milk were contained aflatoxin M1, and the concentration of aflatoxin M1 ranged from 0.25 ppb to 50 pbb, table-2.

TABLE II
QUANTITATIVE DETRMINATION OF AFLATOXIN M1 IN SOME MILK COLLECTED SAMPLES USING INDIRECT ELISA TECHNIQU.

Milk samples	Aflatoxin M1 concentration / ppb
KDD full cream	16.25
KDD half cream	13.75
KDD light cream	5
KDD skimmed	0
Süt aysah	5.5
Alsafi low fat	15
Almarai full cream	5
Almarai half cream	2.5
Almarai light cream	5
Almarai skimmed	10
Nada full cream	25
Nada half cream	50
Nada light cream	37.5
Nadec full cream	50
Nadec half cream	5.5
Nadec light cream	11.25
Pinar	6.25
Milky way	0
Mars	0
Juda	12.5
AL Rai	5
Hammoudeh Banana flavor	12.5
Safio strawberry flavor	0
Safio Banana flavor	2.5
YAG GO vanilla flavor	0.25

Results of the present study show that the concentration of aflatoxin M1 in examined milk samples ranged from 0.25 ppb to 50 ppb, others studies have been conducted in other countries in this context. [17] determined the level of aflatoxin M1 in milk samples ranging from 0.001 to 0.0235ppb. In a study conducted by [18], the mean of Aflatoxin M1 in pasteurized milk was 0.019 ppb. [19] examined 122 samples for aflatoxin M1 and reported that the mean concentration of aflatoxin M1 was 0.04ppb. [20] investigated 85 pasteurized milk samples were analyzed for aflatoxin M1 with the ELISA technique. Seventy-five samples were found to be contaminated with aflatoxin M1and the range of aflatoxin M1 was (27-48 ppb), While [21] revealed that the level of aflatoxin M1 ranging from 0.001 to 0.117 ppb. [22] measured aflatoxin M1in pasteurized milk samples from the School Milk Project in Thailand and The highest concentration of aflatoxin M1 found in school milk samples was 0.114 ppb, whereas [23] revealed that the range of aflatoxin M1 in pasteurized milk ranging from (0.023–0.154 ppb).

U.S Food and Drug Administration (FDA) was determined the action levels of aflatoxin M1 in milk (0.5ppb) [24].

According to FDA´s action levels for aflatoxin M1 in milk and when compared with our results, we can conclude disqualification of the most of milk samples for human consumption due to the concentration of aflatoxin M1 in these samples which exceeded the allowable limit. Detoxification of aflatoxin M1 from milk using UV light. The results of our study is mention in the following table-3:

TABLE III
EFFICIENCY VARIATION OF DETOXIFECATION OF AFLATOXIN M1AT DIFFERENT DISTANCES BETWEEN LAMP AND MILK LAYER, TIME OF EXPOSURE AND VOLUME OF MILK SAMPLE (MILK SAMPLE WAS CHOSEN IN 50 PPB CONCENTRATION)

Distances between lamp and milk layer (cm)	Time of exposure (min)	Volume of milk sample (ml)	Concentration of aflatoxin M1(ppb) (Each value expressed as Mean ± Standard Error (SE) of three replicates
30	15	25	17.5
30	15	50	12.5
30	30	25	17.5
30	30	50	15
60	15	25	15
60	15	50	0.5*
60	30	25	0
60	30	50	0
90	15	25	13.75
90	15	50	0
90	30	25	0
90	30	50	0

* = Significant ($P \leq 0.05$)

From the results that mention in the table above we can observe efficiency of UV light to reduce the aflatoxin M1 from contaminated milk.

Three data were used in our experiment (distances between lamp and milk layer, time of exposure and volume of milk sample). Distance between lamp and milk layer was the most effective parameter in our test, and three distances were used (30, 60 and 90 cm). Results shows that 90 cm distance was more effective, and whenever the distance increase the effect also non-significantly increase, also the other two parameters (time of exposure and volume of milk sample) were effective in reduction of aflatoxin M1 from milk but less than distance parameter.

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