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Comparison between some different methods for determination of Aflatoxin M_1 in milk and some diary products

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Abstract

Seven TLC methods for evaluate the incidence of Aflatoxin M_1 in milk and dairy products were used, Pons *et al*. (1973); Fukayama *et al*. (1980); Stubblefield (1979); Official Method of Analysis (1995); Van Egmond and Stubblefield (1981); Official Method of Analysis (1984) and Official Method of Analysis (1990) , to detect the sensitive method among them for determination of Aflatoxin M1 (AFM1). Results show that, the Official Method of Analysis, 1995 (AOAC, 1995) was the sensitive method for determination of AFM1 among the all tested methods, where it gives the highest recovery percentage for liquid milk, Cheese, and Powdered milk, 106.2%, 95.99% and 104.4% respectively. While Stubblefield, (1979) method gave about 75% AFM1 recovery for Yogurt, but when we made a slight modification on it gave 92% AFM1 recovery.

Introduction

Aflatoxin M_1 is 4-hydroxy-aflatoxin B_1 and Aflatoxin M2 is 4-dihydroxy-aflatoxin B_2. Aflatoxin M_1 appears in milk or dairy products as direct result of the ingestion of feeds contaminated with Aflatoxin B_1 by cattels. The carry-over of AFB_1 also to milk may vary largely from animal to animal, from day to day, and from one milking to the next (Van Egmond and Dragacci, 2001). According to the U.S Food and Drug Administration (FDA), AFM_1 should not exceed 0.5 ppb (Stoloff 1980 and Van Egmond 1989 a,b). Many chromatographic method of analysis have become available for the determination of AFM_1 in milk and milk products. Most of these were developed for the analysis of milk and milk powder, but they can often be used for other dairy products as well, with minor modifications (Stubblefield and Van Egmond, 1990).

The aim of this experiment is to detect the sensitive method among 7 different methods used for determination of Aflatoxin M1 (AFM1)

Material and methods

Evaluation of the TLC Methods Used for the Detection of Aflatoxins in Milk and Dairy Products:

Seven TLC methods were used during the course of the present work to evaluate the incidence of Aflatoxin in milk and dairy products. These methods are indicated as follows:

1- Pons *et al*. (1973).
2-Fukayama et al.(1980)
3-Stubblefield (1979)
4-Official Method of Analysis (1995)
5-Van Egmond and Stubblefield (1981)
6- Official Method of Analysis (1984)
7-Official Method of Analysis (1990)

Extraction Of Aflatoxin In Milk And Cheese

The thin layer chromatography method (AOAC,1995, official method 980.21) was adopted.

a-Extraction (According to AOAC,1995)
b-Column chromatography. (According to AOAC,1995)
c- Thin layer chromatography. (According to AOAC,1995)

Visual analysis (Milk)
(According to AOAC,1995)
Visual Analysis (Cheese):
(According to AOAC,1995)
Densitometric measurements:
(According to AOAC,1995)

3.4. Column chromatography (clean up):
(According to AOAC,1995)

Basic procedure:

Extraction column is held by a simple support bracket over a funnel containing 10 gm granular sodium sulfate in 12 cm filter paper whatman No. 1, and a funnel is placed over a 500 ml round bottom flask. Add 50 ml fluid milk to column and let milk be absorbed. After 5 min, elute AFM_1 with 50 ml chloroform-acetone (9:1). Repeat this extraction step twice. Let solvent drain through column after each addition but don't let column dry between extraction.

Evaporate combined extracts to near dryness on rotary vacuum evaporator, and dissolve residue in 5 ml ethyl ether (anhydrous),being sure to rinse sides of flask. Transfer ether extract to column chromatography containing ca 2gm silica gel deactivated with 3% water and ca 1 gm sodium sulfate layer on top of the silica gel. Rinse flask with 10mL ethyl ether and add rinse to column. Let ether extract drain completely through silica gel column, and discard elute. Elute AFM_1 off column with 10mL chloroform –acetone (9:1) and collect elute in 50 ml round bottom flask. Repeat once. Evaporate extract to near dryness under vacuum and quantitatively transfer with chloroform to 1dram vial stoppered with a teflon-lined screw-cap. Evaporate extract to dryness under steam of nitrogen or water bath and dissolve residue in 100 ul benzene –acetonitrile (9:1) for TLC analysis (Fukayamaet.al.,1980).

3.5. Modification of Stublefield (1979)

50 gm Yogurt, was shaking vigorously with NaCl solution 10 ml (saturated) 35°C and warmed 120 ml CH3CI at 38 °C and mixed with sample and salt solution in separator funnel, mix 1 minute and centrifuging mixture at 4000 rpm for 10 min. The chloroform layer was separated good. Chloroform layer was filtered throw filter paper (Watman No. 1) into a graduated cylinder. The filtrate volume was recorded.

Results and Discussions

Comparison between methods of Pons, *et at*. (1973), Fukayama *et al*. (1980), Stubblefield (1979), Official method of analysis (1995), Van Edmond and Stubblefield (1981), Official method of analysis (1990) and Official method of analysis (1984) were made in milk and some dairy products.

Milk was contaminate with 1µg /L AFM1 (ppb) while Cheese was contaminate with 5 µg /Kg of AFM1 (ppb).

Wel-Yun *et al*. (1984) compared 4 methods of Aflatoxin quantification . Direct TLC of yeast broth showed no significant difference from conventional chloroform extraction, concentration and TLC quantification of Aflatoxins B_1, G_1 and G_2, however , AFB_2 measured by direct TLC method was significantly higher than the other methods used. Methods using silica cartridges for purification or C_{18} cartridges for isolation and purification did not give satisfactory results, when compared to chloroform extraction.

This indicates that the extraction and elution procedures needed to be modified. The direct TLC method proved to be the most economical and rapid method of quantification of Aflatoxins.

Results in table (1) and fig. (1) show that, the Official Method of Analysis, (AOAC, 1995) was the sensitive method for determination of AFM1 among the all tested methods, where it gives the highest recovery percentage for liquid milk, cheese and powdered milk, 106.2%, 95.99% and 104.4% respectively while Stubblefield, 1979 method with slight modification gives the best recovery (92%) for yogurt

Some different artificially AFM1 contaminated (Material and Methods) dairy products, (Liquid Milk, Romi Cheese, Domiati Cheese, Powder Milk and Yogurt) were analyzed for AFM1 content by seven methods as mentioned in material and methods. Our results showed that the method of OAOC (1995) gave the highest recovery percentages of AFM1 content for Liquid Milk, Romi Cheese, Domiati Cheese and Powdered Milk (106.2, 95.99, and 104.4% respectively). On the other hand Stublefield method (1979) gave about 75% AFM1 recovery for Yogurt, while when we made a slight modification on it (Material

and Methods), it gave 92% AFM1 recovery.

Our results were approximately agreement with Stublefield (1979), Pons, *et al.* (1973), Van Egmond & Stublefield (1981) and Fukayama *et al.* (1980).

Stublefield (1979) found that the recovery of AFM1 from artificially contaminated whole raw and powdered milk was 80%.

Pons *et al*. (1973) observed that the average recovery of AFM1 added to fluid milk at levels of 0.1-1.0 µg/L was 106% by using the basic procedure and 90% when the cellulose column was used to purify the extract.

Fukayama *et al*. (1980) tested AOAC 1975,Stublefield 1979 and new method 1980 for determining AFM1 levels in fluid milk. The three quantitative methods gave recovery ranged between 80-90%.

For AFM1 determination in cheese Stublefield (1979) tested different samples to detect the best recovery of AFM1. The recovery ranged between 58% and 100%. Van Egmond and Stublefield, (1981) reported that they method has been used successfully on extracts of milk and cheese containing Aflatoxins M1and B1.

Abd Alla (1983) used Fukayama *et. al*, (1980) and Stublefield, (1979) with centrifugation extraction methods for determine the recovery of AFM1 in yogurt as the similar of milk.

Table (1): Comparison Between Some Different Methods for Determination of Aflatoxin M_1 in milk and some dairy products.

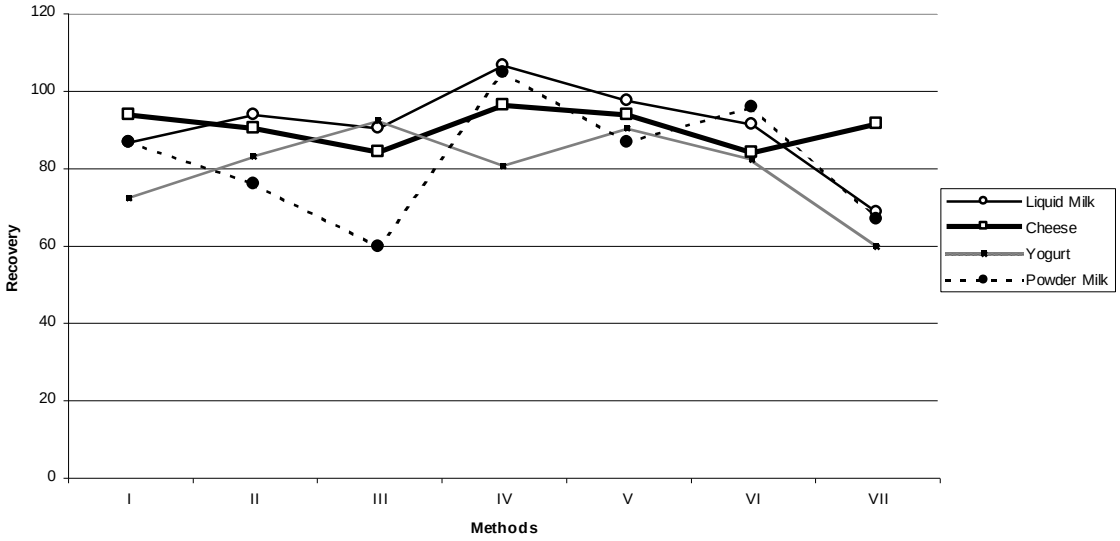
Methods	Liquid Milk		Cheese		Yogurt		Powder Milk	
	Amount of AFM_1 (ppb)	Recovery (%)	Amount of AFM_1 (ppb)	Recovery (%)	Amount of AFM_1 (ppb)	Recovery (%)	Amount of AFM_1 (ppb)	Recovery (%)
I	0.864	86.4	4.679	93.5	0.72	72	0.684	68.4
II	0.936	93.6	4.500	89.99	0.828	82.8	0.756	75.6
III	0.900	90.0	4.200	83.99	0.920	92	0.594	59.4
IV	1.062	106.2	4.800	95.99	0.802	80.2	1.044	104.4
V	0.972	97.2	4.679	93.6	0.900	90.0	0.864	86.4
VI	0.911	91.1	4.190	83.8	0.820	82.0	0.955	95.5
VII	0.684	68.4	4.560	91.2	0.594	59.4	0.666	66.6

I- Pons *et al.(1973).*
II- Fukayama *et al.* (1980).
III- Stubblefield (1979).
IV- Official Method of Analysis (1995).
V- Van Egmond and Stubblefield (1981).
VI- Official Method of Analysis (1990).
VII- Official Method of Analysis (1984).
* AFM_1 Aflatoxin M_1

N.B:

a- 1ppb AFM_1 were added to 1liter of liquid milk, powdered and yogurt
b- 5ppb AFM_1 were added to 1kg.of cheese.

Fig(1): Comparison Between Some Different Methods for Determination of Aflatoxin M_1 in Milk and Some Dairy Products



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تقييم الطرق المستخدمة فى تقدير الأفلاتوكسين م1 فى الألبان ومنتجاتها

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أظهرت النتائج أن طريقة (1995) AOAC كانت أكثر الطرق حساسية وأكفأها فى الإستخلاص بالنسبة للبن السائل والجبن واللبن الجاف بنسب 106.1%، 95.99%، 104.4% على التوالى. فى حين أن طريقة (1979) Stubblefield المعدلة أعطت أعلى نسبة إستخلاص مع اليوجورت (92%) إجراء بعض التعديلات البسيطة عليها.

لذا ينصح بإستعمال طريقة (1995) AOAC فى تقدير الأفلاتوكسين فى اللبن ومنتجاته بينما ينصح باستعمال طريقة (1979) Stubblefield فى اليوغورت مع تسخين اليوغورت 35 درجة مئوية وتسخين الكلورفورم قبل إضافته حتى 38درجة مئوية ثم عمل طرد مركزى على 4000 لفة فى الدقيقة للمساعدة على فصل الدهون وإعطاء نتائج أفضل.