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Mycological Evaluation of Serving Some Dairy Products with Special Reference to Mycotoxins Production in Azhar University Student Hostels

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Abstract: Milk and milk products especially cheeses and yoghurt are essential constituents in daily meals in Student hostels in Azhar University, Egypt. This dairy products may be contaminated with moulds and/or mycotoxins, among mycotoxins aflatoxins are the most dangerous especially aflatoxin B1 which is classified as the potent human carcinogen. The objectives of this study were (i) evaluate dairy products distributed in Azhar University mycologically for incidence, isolation and identification of mould species, (ii) determination the occurrence of aflatoxins in this dairy products and (iii) to compare between Sabouraud dextrose (SDA) and Aspergillus Differentiation Media (ADM) for isolation and counting of *Aspergillus flavus* as a one of most important species which has a public health significant in toxin production. Mycological evaluation was done for 200 random samples of soft cheeses (Feta and Istanboli, 65 each) and 70 plain yoghurt samples distributed in student hostels. About 64.5% of all different samples were positive for mould; 70.7%, 56.9% and 65% of Feta cheese, Istanboli and yoghurt samples respectively. The highest incidences (70.7%) as well as the highest mean (1.50×10^3cfu) were detected in Feta cheese; all the samples were aflatoxin free by HPLC. According to these values, we considered the students in Azhar University are in safe side away from aflatoxicosis.

Key words: Dairy products · Mould · Aflatoxin · Aspergillus Differentiation Medium

INTRODUCTION

Milk has been used by humans since the beginning of recorded as the most popular and nutritious human food, this value put the food hygienists in a real challenge, to provide milk to consumer safe and at the same time keep its nutritional value high as much as possible. In some countries including Egypt most milk is manufactured into more stable dairy products of worldwide commerce, such as cheese and yoghurt. Dairy products manufactured from milk have the same properties of original milk moreover; several stages of processing may be unfavorable and add more points of hazard and weakness allowing entrance of moulds. Unfortunately, these products support mould growth and toxin production due to its contents of protein, low pH and storage under unfavorable conditions [1, 2].

Moulds are microscopic organisms that live on plant or animal matter. No one knows how many species of fungi exist, but estimates range from tens of thousands to perhaps 300,000 or more [3].

Mycotoxins are secondary toxic by-products (not essential for survive) of some moulds produced under certain conditions to achieve a competitive advantage for mould over other mould species and bacteria. It is a heat stable, cytotoxic toxin for all living cells including higher organisms' cells [4].

Mycotoxins produced by certain moulds as a toxic substances can be found in dairy products from two origins: indirect contamination, which results when dairy cows ingest feed that contains mycotoxins which pass into the milk such as aflatoxin M_1 and direct contamination, which occurs because of the intentional or accidental growth of moulds which secrete aflatoxins B1, B2, G1 and G2 [5], therefore, feeding on a low quality ration contaminated with moulds more than 10^6/g and kept under humid conditions cause mycotoxin intoxication to both animals and human whom consumed this animal products.

Therefore our work was adapted to evaluate dairy products distributed in Azhar University mycologically for incidence, isolation and identification of mould

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species, to determine the occurrence of aflatoxins in this dairy products and to compare between Sabouraud and aspergillus differentiation media for isolation and counting of *Aspergillus flavus* as a one of most important species which has a public health significant in toxin production.

MATERIALS AND METHODS

This study included examination of two hundreds random samples of soft cheeses (Feta and Istanboli) (65) each and yoghurt (70) samples which were collected from different student's hostels in Azhar University for mycological evaluation. Each cheese sample was represented by tetra bag packet of 65 g, while sample of yoghurt by cup of 80 g weight.

All samples in this study were collected in the period between October 2012 and May 2013.

The collected samples were transferred directly to the laboratory with a minimum of delay under aseptic condition to be examined mycologically. Each sample was divided into two portions. One for mycological examination and the second for mycotoxins testing for samples shown mould count.

The samples were prepared according the technique reported by APHA [6].

Sabouraud Dextrose agar Chloramphenicol medium (SDA) was used. While, Aspergillus Differentiation agar medium (ADM) was prepared for isolation and giving an indication about *Aspergillus flavus* toxicity [7].

Determination of the total number of mould in cheese and yoghurt samples was conducted in duplicate plates of SDA and ADM according to APHA [6]. The isolated species were identified on the basis of investigation of the macro morphological properties of conidial and other structures according to the key described by Samson *et al.* [8].

Aflatoxin Detection: Thin layer chromatography (TLC) for detection of Aflatoxins preparation and development of TLC plates according to method described in AOAC [9]. After that the quantitative analysis of Aflatoxins B1, B2, G1 and G2 were determined by HPLC after the method of Slubblefield and Shotwell [10].

RESULTS

Fungal Contamination and Aflatoxins Production: Mould was detected in more than 64.5% of all different samples. The highest mean counts as well as the highest positive samples percent were detected in feta cheese while, the lowest mean counts were detected in yoghurt and the lowest incidence of contaminated samples were in istanboli cheese samples (56.9%). About 36% of cheese and 34% of yoghurt samples were complying with Egyptian standards for mould count while 100% for aflatoxins. *Aspergillus*, *Penicillium* and *mucor* genera were more frequently detected than other genera of fungi. Amoung the 2 media used the ADM recovered more isolates of *A. flavus* as shown in Table 3 and Fig.1. All samples of cheese and yoghurt that contaminated by *Aspergillus* species were free of aflatoxins.

DISCUSSION

Dairy products can be contaminated by mycotoxins from two ways first way; indirectly by contamination of animal feed with subsequent passage of the mycotoxins to milk (AFM1) [11] or directly by contaminating dairy products with moulds which multiply during processing, manufacturing and storage and produce aflatoxins (B, B2, G1 and G2); that direct contamination is our research target. Fungal contamination of cheese and yoghurt is a serious threat in the students' hostels especially with the lack of awareness of healthy nutrition and the absence of refrigerator in student rooms. Total mould count is considered an indicator for hygienic condition in dairy plants in many countries [12].

The prevalence and count of mould in Feta and Istanboli cheese and yoghurt samples collected from student hostels of Azhar University had been demonstrated in Table 1. However, it declared that 70.7, 56.9 and 65.7% of Feta cheese, Istanboli and yoghurt samples, respectively are contaminated with moulds. It meant that a total of 64.5% of all different samples were positive for mould. These findings agree with those reported by El-Shrief [13] who detected mould in 27 of 50 samples (54%) fresh Damietta cheese. Montagna *et al*. [14] found Moulds in 54 of the 122 analyzed cheese samples (44.3%). Sabreen and Zaky [15] could detect moulds in 55% of examined soft cheese. El- Asuoty [16] examined 25 yoghurt samples, 9 (36%) of them were mould positive. We obtained a mould count ranged from $1x10^2$ and $7.6x10^3$ cfu/g with a mean of $1.5x10^3$ cfu/g in feta cheese and in Istanboli cheese samples from $1x10^2$ and $5x10^3$ cfu/g with a mean value of $9.9x10^2$ cfu/g. Ghazal [17] analyzed 20 cheese samples (10 of soft fresh and 10 soft brined cheeses) mycologically and found that contamination with moulds in counts

Table 1: Prevalence and statistical analytical results of moulds examination for soft cheese and yoghurt samples

		+ve samples		count cfu/g		
Samples	Examined samples	No.	%	Minimum	Maximum	Mean ±SE
Feta cheese	65	46	70.7	1 x 10^2	7.6 x 10^3	1.5x10^3±0.3248x10^3
Istanboli cheese	65	37	56.9	1 x 10^2	5 x 10^3	9.9x10^2±2.254x10^2
Yoghurt	70	46	65.7	1x10^2	3x10^2	1.5x10^2±0.0791x10^2

Table 2: Comparison between the results obtained from mould examination for examined samples and Egyptian standards (2005)

		Samples comply with Egyptian standard	
Count (cfu/g)	Egyptian standard	Soft cheeses	yoghurt
Mould count	10	47 (36%)	24(34%)

Table 3: Frequency distribution of isolated moulds from examined soft cheeses and yoghurt samples

	Feta		Istanboli		Yoghurt	
Mould spp.	No	%	No	%	No	%
A. flavus	15	24.59	5	12.5	5	9.61
A. niger	14	22.95	9	22.5	9	17.3
A. ochraceous	1	1.63	1	2.5	2	3.84
A. nomius	-	-	1	2.5		
A. terres	-	-	-	-	1	1.92
Acremonium spp.	-	-	-	-	3	5.76
Alternaria	6	9.83	-	-	13	25
Cladosporium spp.	-	-	8	20	6	11.53
Fusarium spp.	-	-	1	2.5	-	-
Geotrichum	-	-	2	5	-	-
Mucor spp.	12	19.67	1	2.5	6	11.53
P. albocormium	-	-	4	10	-	-
P. cammberti	1	1.63	4	10	-	-
P. chrysogenium	-	-	1	2.5	-	-
P. citrinum	-	-	-	-	2	3.84
P. funiculosum	-	-	3	7.5	3	5.76
P. oxalicum	1	1.63	-	-	-	-
P. roquforti	4	6.55	-	-	-	-
P. rubrum	1	1.63	-	-	1	1.92
P. species	5	8.19	-	-	1	1.92
Trichophyton	1	1.63	-	-	-	-
Total	61	100	40	100	52	100

Table 4: Prevalence of *Aspergillus flavus* in examined white soft cheese and yoghurt samples on SDA and ADM Media

			A. flavus samples		
Type	Medium	+ve samples	No.	%	No. of isolates
cheese	SDA	83	18	21.68	20
	ADM	86	22	25.58	26
yoghurt	SDA	46	3	6.52	5
	ADM	51	3	5.88	8

*SDA= Sabouraud Dextrose Agar

*ADM= Aspergillus Differentiation Medium

ranges from 10^3 to 1.05×10^4 cfu /g and 5.5×10^2 to 4.85×10^3 cfu/g, respectively. Bullerman [18] found similar results in cheese (10-7.5x10^2cfu/g). Arizcun *et al*. [19] declared total mould count less than 10^3cfu/g while, yoghurt maximum count was 3x10^2 cfu/g and mean value 1.5x10^2 cfu/g.

The results in Table 2 indicated that only 47 (36%) of examined white soft cheese and 24 (34%) of plain yoghurt comply with Egyptian standard [20, 21]. Presence of moulds in cheese and yoghurt reflect unsanitary hygienic measures in manufacturing and packaging [22, 23].

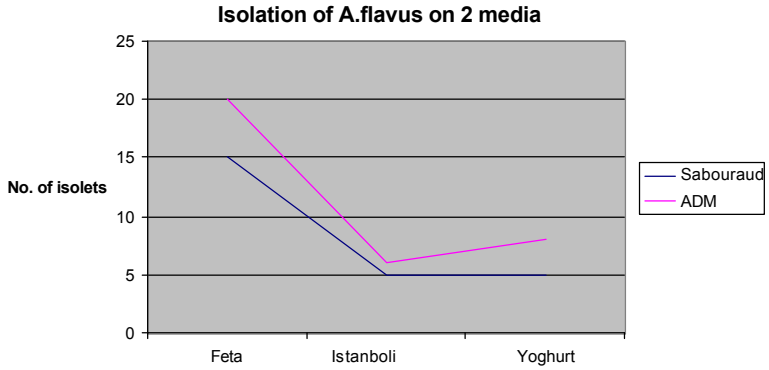

Fig. 1: Prevalence of *Aspergillus flavus* in examined white soft cheese and yoghurt samples on SDA and ADM media.

From the aforementioned results it is obvious that the mould contamination levels in Feta cheese were higher than that found in Istanboli cheese and yoghurt. This may be due to the low salt concentration (5%) in Feta cheese associated with long shelf life which expanded to 12 months comparing to 14 days for yoghurt which are processed from sterile milk which destroy huge proportions of mould populations. Also high salt contents (6%) of Istanboli cheese may act as mould suppressor.

Identification of isolated mould species revealed that *Aspergillus*, *Acremonium, Alternaria, Cladosporium, Fusarium, Geotrichum, Mucore* and *Penicillium* ontaminate the samples. El-Diasty and El-Kaseh [24] also isolated *Aspergillus spp., Cladosporium spp., Mucor spp., Curvularia spp., Penicillium spp., Geotricum spp.* from milk and yoghurt. In this study *Aspergillus*, *Mucor* and *Penicillium* were the most dominant in the contaminated samples. These results are in agreement with that reported by El-Asuoty [16] Ghazal [17] and Berikten. and Kivanc [25] while, in some studies [26, 14] *Penicillium* species were the most frequently isolated followed by *Geotrichum* spp. (7.3%), *Aspergillus spp*. (4.2%) and *Mucor* spp. (4.2%). Otherwise, Allaraj *et al.* [27] recorded *Aspergillus spp, Penicillium spp, Fusarium* and *Mucor* as the most common moulds found in analyzed cheeses. Among isolated *Aspergillus spp*. *A. niger* and *A. flavus* were the major isolates (22.59, 22.5and 17.3%) for *A. niger* and (24.5, 12.5 and 9.6%) in Feta, Istanboli and yoghurt samples, respectively, as shown in Table 3. These findings are similar to those reported by El- Asuoty [16], Gazal [17] and Abo Donia [28].

Mould contamination may represent a great hazard even in small counts if the package is opened and kept under uncontrolled temperature and moisture conditions which activate mould to produce toxins [29]. Moulds must be prevented from reach to food and food commodities especially milk as a vital step in prevention of mycotoxicosis. This can be achieved through, avoid contaminated feeds by more than 10^4/ g from animal feed, application of restricted hygienic measures in dairy plants and preserving opened milk products in refrigerator.

In comparing the efficiency of SDA and ADM, ADM showed high recovery rate of *A. flavus* as declared in Table 3 and Fig. 1 as reported by Cotty [30]. Fourteen (30.04%) of examined Feta cheese samples were contaminated with *A. flavus* and 15 isolates were recovered on SDA, while 16 (32.5%) samples and 20 isolates were recovered on ADM. also Istanboli cheese samples reflected 4 (10%) with 5 isolates and 6 (16.22%) with 6 isolates and yoghurt samples contained 5 and 8 isolates of *A. flavus* on SDA and ADM, respectively.

Samples contaminated by *Aspergillus flavus* were screened by TLC and HPLC and all of samples were free of aflatoxin. It reflects that the presence of *Aspergillus flavus* in food does not mean necessarily the presence of mycotoxins and vice versa [31]. Cano-Sancho *et al*. [32] analyzed cheese and yoghurt samples for aflatoxin and could not detect any occurrence of aflatoxin in cheese samples and only 2 of 72 yoghurt samples were contaminated but in acceptable limit according to legal EU limits. Positive cheese samples for AFM1 were recorded in the study of Montagna *et al*. [33], 16.6%, in medium term (19.3%) and long term ripened cheeses (17.1%), they returned this to the high affinity of AFM_1 for milk proteins and therefore during the cheese-making process ripening does not reduce the levels of AFM_1 but on the contrary increases them, due to water loss. Higher results were recorded by Kokkonen *et al.* [34].

In conclusion, the obtained results showed high contamination of white soft cheese and yoghurt with different types of moulds which constitute a public health hazard. Storage of food under condition which prevents mould growth and strict hygienic measures and regulations should be imposed during processing, packaging and transportation.

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