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MYCOTOXIGENIC FUNGAL CONTAMINANT OF PROCESSED CHEESE AND DRIED MILK

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ABSTRACT

A total of one hundred and twenty random samples of processed cheese and dried milk were examined for incidence of mycotoxigenic moulds.

The result show that processed cheese samples contained 95.7% moulds with a mean count of $2.25x10^3$ cfu/g while 78% of dried milk samples had mould counts with a mean value of $3.69x10^2$ cfu/g.

Aspergillus, Penicillium, Fusarium, Alternaria, Cladosporium, Paecilomyces and Mucor was isolated from the examined samples. Aspergillus and Penicillium species predominate all species isolated.

Aspergillus spp. were isolated from 34.3% of processed cheese samples and identified as A. niger (17.9%), A. candidus (7.5%), A. flavus (6%), A. ochraceus (6%), A. wentii (3%), A. versicolour (1.5%), A. terres (1.5%), A. penicilloid (1.5%) and A. fumigatus (1.5%), while Asperigillus spp. isolated from 17.94% of dried milk samples were identified as A. niger (5.1%), A. flavus (10.3%) and A. fumigatus (2.7%).

Moreover, Penicillium spp. were isolated from 11.9% of processed cheese samples and identified into P. verrucosum, P. expansum, P. rogueforti and P. camemberti by incidence percentage of (3%) for each, while Penicillium spp. were detected in 28.2% of dried milk samples and identified as P. verrucosum in (28.2%), P. chrysognum (5.1%) and P. Expansum (2.6%) of examined samples.

A total of eleven A. flavous strains were screened for their ability to produce aflatoxin B_1 in liquid synthetic medium (YESB) and viewed visually on TLC plates. Three strains were toxigenic, one from processed cheese and two from dried milk samples.

The public health importance of moulds and alfatoxins as well as recommendations concern with production, handling and storage of dairy products were discussed.

INTRODUCTION

The production of wholesome milk of good keeping quality is a matter which depends largely on the dairy farmer and his staff, which must possess a sound knowledge of the science of cleaning ***(Doyle, et al., 2001).***

Cheeses and dried milk are manufactured from milk through several stages of processing which at time may be unfavorable and add more points of weakness allowing entrance of moulds. Unfortunately, these products support mould growth and toxin production. Moreover, moulds resist low water activity of dried milk and use protein of cheese as energy source ***(Gqualeni, et al., 1997*** and ***Pardo et al., 2004).***

The mycotoxin producing fungi are mostly related to three main genera, Aespergillus, Fusarium and Penicillin ***(Sweeny and Dobson, 1999)*** and Aspergillus spp. Are certainly the most important species as *A. flavus* and *A. parasiticus* produce the more potent mycotoxins known aflatoxins which are of the greatest significance in food.

Penicillium species have the ability to produce wide ranges of toxic compounds. Also Fusarium species can contaminate man and animal feed/food and produce toxins which are favored by undulating, warm-cool temperatures ***(C.A.B., 1971)***.

Therefore, this work was planned to emphasize informations on the mycotoxigenic fungal contaminants of processed cheese and dried milk samples.

MATERIAL AND METHODS

A total of one hundred and twenty random samples of processed cheese (70 samples) and dried milk (50 samples) were collected from different groceries and supermarkets and pharmacies in Dakahlia governorate for mycological examination.

Representitive samples were transferred directly to the laboratory in their original packages with a minimum of delay under aseptic conditions then prepared according to the technique reported by ***APHA (1992)*** as follow:

Processed cheese:

Eleven grams from each sample were weighted and aseptically homogenized with 99 ml of sterile 2% sodium citrate solution, using sterile electric mixer for two minutes to form a dilution of 10^{-1}, from which, ten-fold serial dilutions were prepared.

Dried milk:

The original dilution was made by reconstituting eleven grams from each can with 99 ml of sterile distilled water and mixed to form a dilution of 10^{-1}, from which decimal dilutions were prepared.

Mycological examination:

100 µL from each dilution was spread onto duplicate plates of sabouraud dextrose agar (SDA) medium. On the same time, 100 µL from each dilution was spread onto Aspergillus differentiation agar (ADM) medium.

The inoculated plates as well as the control one were incubated at 25ºC for 3-5 days.

The first examination was done after 3 days to determine the degree of mould growth (star shape). After 5 days countable plates were selected, counted and different mould growth were picked up and transferred to Czapek yeast extract agar (CYA) slants, and incubated at 25ºC for 3 days for further identification.

On the other hand, the orange yellow coloured base colonies on Asperigillus differentiation agar medium (ADM) which are characteristic for *A. flavus* colonies were picked up after two days onto (CYA) slants and incubated for further identification.

Identification of isolated moulds:

Each isolated mould was inoculated by using sterile needle onto a duplicate plates of Czapek-Dox agar and Malt extract agar media and incubated for 3-5 days at 25ºC.

The growth was identified macroscopically for colour, size, pigment, texture and reveres and microscopically for conidia, conidophore, phialids and head according to ***Raper and Fennell (1965)*** for the genus Aspergillius, and according to ***Ramirez, (1982)*** for the genus Penicillium, while other genera were identified according to ***Barnnett and Hunter, (1972).***

The screening of *Aspergillius flavus* strains isolated for aflatoxin B_1, production was done as suggested by ***DAVIS, et al., (1966)***.

RESULTS AND DISCUSSION

Table (1): Incidence and count of moulds in the examined samples:

Types of examined samples	No. of examined samples	Positive samples		Count (cfu/g)		
		No.	%	Min.	Max.	Mean ± SE*
Processed cheese	70	67	95.7	$1x10^2$	$8x10^3$	$2.25x10^3 \pm 0.263x10^3$
Dried milk	50	39	78.0	$1x10^2$	$2.6x10^3$	$3.69x10^2 \pm 0.74x10^2$

*SE = standard error.

Table (2): Mould genera isolated from positive samples.

Mould genera	Processed cheese (No 67)*		Dried milk (No. 39)*	
	No of positive samples	%**	No. of positive samples*	%**
Aspergillius	23	34.3	7	17.94
Penicillium	8	11.9	11	28.2
Fusarium	2	2.9	-	-
Other genera: -Cladosporium -Alternaria - Paecilomyces -Mucor	50	74.6	30	76.9

***No =** number of positive samples.

****% =** percentage were calculated in relation number of samples contained moulds (67) and (39) respectively.

Table (3): Aspergillus species isolated from processed cheese and dried milk samples.

Type of samples	Aspergillus species	Frequency	
		No.*	%**
processed cheese	*A. niger*	12	17.9
	A. candidus	5	7.5
	A. flavus	4	6.0
	A. ochraceus	4	6.0
	A. wentii	2	3.0
	A. versicolour	1	1.5
	A. terres	1	1.5
	A. pencilloid	1	1.5
	A. fumigatus	1	1.5
Dried milk	*A. niger*	2	5.1
	A. flavus	4	10.3
	A. fumigatus	1	2.7

No* = number of positive samples.

%** = percentages from positive samples (67) and (39) respectively.

Table (4): Pencillium species isolated from processed cheese and dried milk samples.

Type of samples	Pencillium species	Frequency	
		No.	%*
processed cheese	P. verrucosum	2	3.0
	P. expansum	2	3.0
	P. roqueforti	2	3.0
	P. camemberti	2	3.0
Dried milk	P. verrucosum	11	28.2
	P. expansum	1	2.6
	P. chrysognum	2	5.1

%* = percentages were calculated in relation to the number of contaminated samples (67), (39) respectively.

Table (5): Number of aflatoxigenic strains of A. *flavus* isolated from examined samples.

Type of samples	No. of isolated A. flavus strains	Alfatoxigenic strains	
		No.	%
Processed cheese	5	1	20
Dried milk	6	2	33.3
Total no.	11	3	27.27

(A) The total mould counts:

The results achieved in table (1) declare that the moulds could be isolated from 95.7% and 78% of processed cheese and dried milk samples respectively. Mould counts range from $1x10^2$ to $8x10^3$ with a mean value of 2.25×10^3 cfu/g were counted from processed cheese and $1x10^2$ to 2.6×10^3 cfu/g with a mean value of 3.69×10^2 cfu/g from dried milk.

From the aforementioned results it is obvious that the degrees of mould contaminations in processed cheese were higher than that found in dried milk. This may be due to the unhygienic manufacturing practices and exposure of cheese during processing and packaging to contamination from air, brine tanks and shelves ***(Frazier, 1976),*** while dried milks are packaged and processed under controlled methods of sterilization which destroy great proportions of mould populations.

(B) Isolated mould genera:

Aspergillus species were isolated from 23 (34.3%) of processed cheese samples while 8 samples (11.9%) of processed cheese had Penicillium spp. and 2 (2.9%) had Fusarium spp. (Table, 2).

These findings declared that Aspergillus spp. predominate mould species in processed cheese where the chance for aflatoxin production permits. On the other hand, Penicillium spp. were isolated from 11 (28.20%) dried milk samples. Aspergillus spp. From 7(17.49%) dried milk samples, while Fusarium spp. failed to be detected in any dried milk samples examined.

Other moulds such as Cldosporium, Alternaria, Paecilomyces and Mucor were isolated from 50 (74.6%) processed cheese samples and from 30 (76.9%) dried milk samples.

Contamination of milk by moulds play a significant role in spoilage of cheese and dried milk made from such milk. Many moulds like Aspergillus, Penicillium and Fusarium may find the opportunity to grow and multiply in the product and producing toxic metabolites like mycotoxins ***(Ramiriz, 1982; Pit and Hocking, 1985 and El-Deeb et al, 1992)***.

Some Penicillium spp. has a lipolytic activity and was found to be the causative agent of rancidity in dairy products.

(C) <u>Isolated Aspergillus species</u>:

The most predominant mould species was *A. niger* 12 (17.9%) in processed cheese samples followed by *A. candidus* 5 (7.5%), *A. ochraceus* 4 (6%), *A. flavus* 4 (6%), *A. wentii* 2 (3%), *A. versicolour* 1 (1.5%), *A. terres* 1 (1.5%), *A. penicilliod* 1 (1.5%) and *A. fumigatus* 1 (1.5%) (Table, 3).

The contaminations of examined samples by Aspergillus species lead to spoilage of the appearance as well as, they produce lipolytic and proteolytic enzymes. Besides, aflatoxins may be produced in dairy products kept at the ambient temperature (25ºC) of storage due to the growth of toxic strains of *A. flavus*.

(D) Isolated Penicillum species:

The prevalence of Penicillium spp. in the examined samples is reported in table (4).

Each of *P. verrecosum, P. expansum, P. roqueforti* and *P. camemberti* was isolated from 2 (3%) of examined processed cheese samples, while *P. verrucosum* was isolated from 11 (28.2%) followed by *P. chrysognum* 2 (5.1%) and *P. expansum* 1 (2.6%) of dried milk samples.

The presence of Penicillum species in examined samples indicates rapid spoilage of dairy products, as well as production of bad mouldy odours and exudates which reduce the acceptability of products to consumers. Pencillia even if not responsible for food poisoning by itself but under favorable conditions can produce some mycotoxins that may lead to public health hazards.

Although, aflatoxin, a highly potent carcinogen produced by certain strains of *A. flavus*, however, mycotoxin production is not limited to aflatoxigenic moulds, with certain of strains of Alternaria, Aspergillius, Cladosporium, Fusarium, Mucor and Pencillium isolated from cheese also being capable of synthesizing toxins ***(Scott, 1989)***.

(E) Screening of aflatoxin producing A. flavus strains:

The data reported in table (5) reveal that (20%) and 2(33.3%) of *A. flavus* strains isolated from processed cheese and dried milk samples respectively, could produce AFB_1.

Aflatoxigenic species are the most importance from the heath point of view. So the interest in screening *A. flavus* strains isolated from examined samples for the ability to produce aflatoxins has been increased. Also, there is an association between hepatitis C virus (HCV)

and aflatoxin exposure studies by ***Abd-Allah et al., (2003)*** on Egyptian patients. So, the eleven *A. flavus* strains isolated were tested qualitatively in a liquid synthetic medium (yeast extract sucrose broth) by thin layer chromatography (TLC) technique for aflatoxin B_1 production.

Avoid feeding of lactating cows on mouldy feeds, avoid keeping dairy products out of refrigerator, addition of harmless mould inhibitors and applying a strict hygienic conditions in the diary plant are considered as a suggestive measures should be applied to avoid food from contamination by moulds and to safe the consumers from the danger of mycotoxins.

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الملوثات الفطرية المفرزة للسموم في الجبن المطبوخ والحليب المجفف

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****قسم الرقابة الصحية على الأغذية – كلية الطب البيطري – جامعة كفر الشيخ**

أجريت الدراسة على مائة وعشرين عينة من منتجات الألبان، سبعون من الجبن المطبوخ، وخمسون من اللبن المجفف، جمعت من أماكن مختلفة بمدن الدقهلية، تمثل مختلف المستويات الفطرية، لضمان تحقيق أكبر قدر من الدقة، وذلك لتقرير حالتها الصحية الميكولوجية.

وقد أسفرت الدراسة عن تحديد عدد العفن في عينات الجبن المطبوخ وكانت بنسبة 95.7% بمتوسط عددي 2.25×10^3/جم، وفي عينات اللبن المجفف بنسبة 78% بمتوسط عددي 3.69×10^3/جم.

وقد أمكن عزل وتصنيف أجناس العفن من عينات الجبن المطبوخ وكانت كالآتي: الأسبرجلس Aspergillus والبنسليوم Penicillium والفيوزريوم Fusarium بنسب 34.، 11.9، 2.9% على التوالي وكذلك عفن كلادوسبوريم Cladosporium وألترناريا Alteranaria وباسيلومايسس Paecilomyces وميوكر Mucor بنسبة 74.6%.

وبالنسبة للحليب المجفف أمكن عزل أجناس اسبرجلس Aspergillus وبنسليوم Penicillum بنسبة 17.9 ، 28.2% علي التوالي، وعفن كلادوسبوريم Cladosporium وألترناريا Alternaria وباسيلومايسس Baecilomysis وميوكر Mucor بنسب 76.9%.

وقد تم عزل وتصنيف عترات عفن أسبرجلس نيجر *A.niger* واسبرجلس كانديدس *A.candidus* وأسبرجلس فلافس *A. flavus* وأسبرجلس بنسيليود *A.penciolid* وأسبرجلس فيومجاتس *A. fumigatus* من عينات الجبن المطبوخ بنسبة 17.9، 7.5، 6، 1.5، 1.5، 1.5، 1.5% على التوالي.

أما عينات اللبن المجفف فتم عزل وتصنيف عفن الأسبرجلس نيجر *A.niger* واسبرجلس فلافس *A.flavus* وأسبرجاس فيومجاتس *A.fumagatus* بنسبة 5.1، 10.3، 2.7% على التوالي.

أمكن عزل وتصنيف عترات عفن بنسليوم فريكوزم *P.verrecosum* وبنسليوم أكسبينسم *P.expansum* وبنسليوم روكفروتي *P.requeforti* وبنسليوم كاممبرتي *P.camemberti* بنسبة 3% لكل منها من عينات الجبن المطبوخ، وقد تم عزل بنسليوم فريكوزم *P.verrecosum* وبنسليوم اكسبينسم *P.expansum* وبنسليوم كريزوجنيم *P. chrysognum* من 28.2، 2.6، 5.1% من عينات اللبن المجفف على التوالي.

وقد تم اختيار إحدى عشرة عترة من عفن اسبرجلس فلافس *A.flavus* للتحقق من قدرتها على إفراز الأفلاتوكسن، فتبين أن ثلاث منها (27.27%) واحدة (20%) من عينات الجبن المطبوخ، واثنين (33.3%) من عينات الحليب المجفف، قادرة فعلاً على إفراز سم افلاتوكسن ب1.

وقد تم مناقشة الأهمية الصحية للعفن المعزول وإفرازه لسموم الأفلاتوكسين من عينات الجبن المطبوخ والحليب المجفف، وكذلك الأهمية الاقتصادية، مع عرض التوصيات والطرق التي يجب إتباعها لمنع أو تقليل تواجد العفن في الجبن المطبوخ والحليب المجفف عند الإنتاج والتصنيع والتداول والتخزين لتلك المنتجات اللبنية.