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*Article*:

# Detection of Molds in Egyptian Ras Cheese with Special Reference to *Aspergillus flavus* Aflatoxins

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## Abstract

Ras cheese is the most common hard cheese in Egypt. It is usually produced from raw cow milk and ripened in unhygienic conditions; this may promote the growth of molds. This study was conducted to determine the contaminant molds in different samples of Egyptian Ras cheese and detect the level of fungal toxins as AFM1 and AFB1 in positive *Aspergillus flavus* samples. A total of 150 Ras cheese samples (recent, medium and old, 50 for each) were collected randomly from different dairy shops and supermarkets, located in Sohag Governorate, Egypt. These samples were examined for total mold counts. The results revealed that the mean total mold count in recent, medium and old Ras cheese samples were $41\times 10^5 \pm 14\times 10^5$, $12\times 10^6 \pm 1.9\times 10^6$ and $48\times 10^6 \pm 5.9\times 10^6$ CFU/g, respectively. All samples were exceeded the permissible limits of total mold counts established by Egyptian standard (ES). *Aspergillus* was the most predominant genus and represented by eight species namely *A. flavus, A. niger, A. fumigatus, A. nidulans, A. brasiliensis, A. ochraceus, A. parasiticus* and *A. terreus*. The incidence of *A. flavus* samples in recent, medium and old Ras cheese samples were 22, 25 and 37, respectively. All positive *A. flavus* samples were exceeded the permissible limits of AFB1 established by Egyptian Standard (ES). Also, 58.3 % of positive *A. flavus* samples were exceeded the permissible limits of AFM1 established by Egyptian Standard. Therefore, Ras cheese should be made from pasteurized milk to avoid mold contamination and their mycotoxins.

**Keywords:** Aflatoxin B1, Aflatoxin M1, *Aspergillus flavus*, Egyptian Ras Cheese (Romy), Mold.

## Introduction

Ras cheese (Romy) is the most popular traditional hard cheese in Egypt, it is favorable due to its taste and rich nutrient values. Ras cheese accounts for 20% of total cheese production in Egypt [1]. This kind of cheese is made from a mixture of cow's and buffalo's milk or solely from raw cow's milk, and marketing when it has a pungent sharp flavor after 3 to 6 months [2]. It is a rich source of calcium and protein. It also contains large quantities of vitamins A, $B_{12}$, zinc, and phosphorus which are essential to the body's health because of their critical role in building the bones and teeth and providing the brain with essential fatty acids [3]. It is the most preferred and easy food for children and adults in Egypt, especially as a school sandwich or a quick breakfast, so attention to the healthy product of the Ras cheese is very important for the human health [4]. According to the recent trend, all dairy products are supposed to be manufactured from pasteurized milk, so the Egyptian Standards (1007-5/2005) specify that Ras cheese should be made from pasteurized milk and should contain not less than 60% solids and 45% fat in

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solids, and the final product must be free from pathogenic microorganisms [3]. A common issue for the cheese manufacturers during the ripening and curing, as well as for retailers and consumers during refrigerator storage, is fungus growth [5]. Ras cheese is sometimes stored in humid and unsanitary conditions that support the growth of yeasts and molds. Mold growth on cheese poses a serious risk to food safety and quality as well as significant economic losses [6]. Fungi are significant spoilage microorganisms of foodstuffs during storage, since they have a great versality for growing substrates and conditions where other microorganisms are not able to grow. This makes the food unfit for human consumption by reducing its nutritive value and occasionally by producing mycotoxins [7]. Fungi responsible for problems in cheese production are diverse and belong to several genera as *Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium* and *Penicillum* [8]. However, the genus which most frequently isolated from spoiled cheese is *Penicillium* followed by *Aspergillus* [9]. Molds of the *Aspergillus* genus is particularly dangerous in two ways: they are opportunistic infections that can cause aflatoxicosis from the development of aflatoxin in food products, as well as aspergillosis and allergies of the respiratory system. [10]. *A. flavus* is well known to be the main producer of carcinogenic aflatoxins. The existence of this fungus and its toxins is of great importance in terms of food safety where they can produce aflatoxins in several points of their production chain [11]. Aflatoxins are innately fluorescent compounds, especially those four major aflatoxin classes- aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Among these types of aflatoxin, AFB1 is the most potent toxin and carcinogen than others in humans and animals. The reason for its existence in milk and milk products could be that the cow liver partially converted feedstuffs containing AFB1 into AFM1, which is then secreted in milk [12]. While Aflatoxin M1 and M2 are produced as metabolites of B1 and B2, and they are commonly found in milk [13,14]. The International Agency for Research on Cancer (IARC) has kept AFB1 under “group I” due to its high toxicity, teratogenicity, hepato-carcinogenicity and mutagenicity [15]. Aflatoxin M1 was also categorized as "group I" by the IARC in 2012 based on its toxicity and carcinogenicity [16]. Egyptian Organization for Standardization and Quality Control stated that AFB1 should be absent in milk and dairy products, while maximum permissible limit of AFMI is 50 ng/kg in milk, in accordance with the EOSQC guidelines [17] and EC regulation [18] while the US Food and Drug Administration recorded that the permissible limit of AFM1 is 500 ng/kg [19]. This study investigated the mold contamination of Egyptian Ras cheese and their aflatoxins

## Materials and Methods

### Ethical approval

There is no need to ethical approve because the samples weren`t animals but Ras cheese.

### Collection of samples:

A total of 150 Ras cheese samples (Recent, Medium and Old; 50 for each) were collected randomly from different dairy shops and supermarkets, located in Sohag Governorate, Egypt; at the period of April 2022 to March 2023. Each sample was divided into two parts with a clean sterile knife under strict hygienic conditions, the first part was prepared for mycological examination and the second part was used in aflatoxins detection. Samples were prepared according to APHA [20].

### Enumeration of total molds in Ras cheese samples

From each sample ten-fold serial dilutions were prepared, then spread 0.1 ml from each dilution over a dry surface of Sabaroud Dextrose Agar (SDA) plates “obtained from HiMedia, India” containing chloramphenicol and vancomycin (0.05g/ L) using a bent glass rod till complete absorption of the inoculum. The inoculated plates were incubated in a cooled incubator at 25°C for 5-7 days. Then single colonies of fungi developed on SDA medium were counted, picked up and maintained on SDA slopes which were incubated at 25°C for 5 days for further identification [20].

### Phenotypic identification of the isolated mold

Mold colonies were identified by examining their macroscopic and microscopic features according to Domsch et al. [21] for fungi in general, Pitt [22] for *Penicillium* species, Raper and Fennell [23] for the genus *Aspergillus*, Leslie and Summerell [24] & Ismail et al. [25] for *fusarium* species, and Simmons [26] for *Alternaria* species.

### Detection of Aflatoxin M1 & Aflatoxin B1 in Ras cheese samples

Quantitative analysis to evaluate the presence of AFB1 and AFM1 in the different Ras cheese samples was performed by competitive enzyme immunoassay [27], using REF AFB1 kits (immunospec Corporation, California, USA; cat no. SG-4023) and AFM1 kits (immunospec Corporation, California, USA; cat no. SG-4033) in Molecular Biology Researches & Studies Institute, Assiut University.

### Statistical analysis

The statistical microbiological data analysis was prepared by using Excel software version 2019 and SPSS program (SPSS inc., Chicago, IL, USA).

## Results

Realizing the data outlined in **Table 1**, it was evident that 49 of the 50 recent Ras cheese samples were contaminated with mold. The level of contamination ranged from $32\times10^2$ to $50\times10^6$ CFU/g with a mean count of $41\times10^5 \pm 14\times10^5$. All medium & old Ras cheese samples were contaminated with molds and the level of contamination ranged from $10\times10^5$ to $75\times10^6$ & $40\times10^5$ to $148\times10^6$ CFU/g with a mean count of $12\times10^6 \pm 1.9\times10^6$ & $48\times10^6 \pm 5.9\times10^6$ CFU/g, respectively.

As mentioned in **Table 2.** high percentage of contamination with molds in recent & medium Ras cheese samples within the range of $10^6 \geq 10^7$ CFU/g in 61.22% and 56%, respectively. While the high percentage of contamination with molds in old Ras cheese samples within the range of $10^7 \geq 10^8$ CFU/g in 74%.

Based on the findings presented in **Table 3.**, a total of 123, 192 &300 mold isolates were detected in recent, medium and old Ras cheese samples, respectively. The classification position of obtained mold isolates was classified in seven families *(Microascaceae, Trichocomaceae, Nectriaceae, Pleosporaceae, Trichosphaeriaceae, Didymellaceae* and *Davidiellaceae*), 7 genera (*Aspergillus, Penicillium, Fusarium, Alternaria, Nigrospora, Epicoccum* and *Cladosporium*). *Aspergillus* where the most predominant mold genus isolated from different Ras cheese samples, followed by genus *Cladosporium, Fusarium & Penicillium.*

Inspection the results in **Figure 1.** showed that in Ras cheese samples, several species of *Aspergillus* as *A. flavus, A. fumigatus, A. nidulans, A. brasiliensis, A. niger, A. ochraceus, A. parasiticus* and *A. terreus* were isolated in percentages of 56, 32.7, 17.3, 16.7, 34, 10, 46 and 14.7%, respectively. While *Penicillium* species as *P. pinophilum, P. oxalicum, P. bilaii and P. crustosum* were present in percentages of 15.3, 4, 6.7 and 8.7%, respectively. Meanwhile, *Fusarium* species such as *F. incarnatum, F. nisikadoi,* were detected in percentages of 26.7 and 10%, respectively. Otherwise, *Alternaria alternate, Nigrospora oryzae, Epicoccum nigrum* and *Cladosporium cladosporioides* were isolated from Ras cheese samples with percentages of 18.7, 35.3, 16.7and 40.7%, respectively.

The incidence of *A. flavus* was higher in old Ras cheese samples with a percentage of 74% than in recent and medium Ras cheese samples with percentages of 44 and 50%, respectively **Table 4.**

In the present study, we found that all positive *A. flavus* samples in different types of Ras cheese samples were positive for AFB1. The levels of contamination with AFB1 ranged from 2.12 to 2.59, 0.87 to 2.64 and 0.74 to 2.62 ng/kg with an average of 2.46 ± 0.03, 2.37 ± 0.06 and 2.44 ± 0.05 ng/kg in recent, medium & old Ras cheese samples, respectively according to **Table 5.** Comparing the detected levels of AFB1 in examined Ras cheese samples with Egyptian Standards, the European Commission and US FDA which stated that AFB1 should be absent in milk and dairy products, we found that all positive *A. flavus* samples exceed the permissible limits of AFB1 **Table 6.**

All positive *A. flavus* samples in different types of the Ras cheese samples were positive for AFM1. The level of contamination with AFM1 ranged from 9.1 to 236.2, 14.65 to 287.2 and 8.86 to 527.7 ng/kg with an average of 79.7 ± 13.49, 87.25 ± 14.71 and 102.34 ± 17.24 ng/kg in recent, medium & old Ras cheese samples, respectively **Table 7.**

Our study showed that all positive *A. flavus* samples in recent & medium Ras cheese agreed with the mentioned limit of the US regulations (500 ng/kg), while one old Ras cheese sample (2.7%) was exceeding the permissible limit according to US regulations. In contrast, 59.1%, 56% and 59.46% of recent, medium and old Ras cheese samples, respectively were unacceptable i.e. exceeded the prescribed safety limits of the Egyptian regulation and the EC regulation (50 ng/ kg) **Table 8.**

***Table 1 Statistical analytical results of total mold counts/g recovered from examined Ras cheese samples.***

| Types of Ras cheese samples | Total No. of examined samples | Positive samples | | (CFU/g) | | |
|---|---|---|---|---|---|---|
| | | No. | % | Min | Max | Mean±SE |
| Recent | 50 | 49 | 98 | $32\times10^2$ | $50\times10^6$ | $41\times10^5 \pm 14\times10^5$ |
| Medium | 50 | 50 | 100 | $10\times10^5$ | $75\times10^6$ | $12\times10^6 \pm 1.9\times10^6$ |
| Old | 50 | 50 | 100 | $40\times10^5$ | $148\times10^6$ | $48\times10^6 \pm 5.9\times10^6$ |

***CFU = Colony-forming unit, SE = Standard error***

***Table 2 Frequency distribution of mold count in examined Ras cheese samples***

| Counts/ gm | Type of Ras cheese samples | | | | | |
|---|---|---|---|---|---|---|
| | Recent | | Medium | | Old | |
| | No./49 | % | No./50 | % | No./50 | % |
| **$10^2$ -** | – | – | – | – | – | – |
| **$10^3$ -** | 1 | 2.04 | – | – | – | – |
| **$10^4$ -** | 2 | 4.08 | – | – | – | – |
| **$10^5$ -** | 13 | 26.53 | – | – | – | – |
| **$10^6$ -** | 30 | 61.22 | 28 | 56 | 3 | 6 |
| **$10^7$ -** | 3 | 6.12 | 22 | 44 | 37 | 74 |
| **$10^8$ -** | – | – | – | – | 10 | 20 |
| **Total** | 49 | 100 | 50 | 100 | 50 | 100 |

***Table 3 Frequency distribution of different mold genus in different types of Ras cheese samples***

| Type of Ras cheese samples | No. of positive samples | No. of total isolates | Family | Genus | | |
|---|---|---|---|---|---|---|
| | | | | Type | No. | % |
| Recent | 49 | 123 | *Microascaceae* | *Aspergillus* | 75 | 60.97 |
| | | | *Trichocomaceae* | *Penicillium* | 10 | 8.13 |
| | | | *Nectriaceae* | *Fusarium* | 9 | 7.31 |
| | | | *Pleosporaceae* | *Alternaria* | 6 | 4.87 |
| | | | *Trichosphaeriaceae* | *Nigrospora* | 8 | 6.50 |
| | | | *Didymellaceae* | *Epicoccum* | 4 | 3.25 |
| | | | *Davidiellaceae* | *Cladosporium* | 11 | 8.94 |
| Medium | 50 | 192 | *Microascaceae* | *Aspergillus* | 107 | 55.73 |
| | | | *Trichocomaceae* | *Penicillium* | 17 | 8.85 |
| | | | *Nectriaceae* | *Fusarium* | 18 | 9.37 |
| | | | *Pleosporaceae* | *Alternaria* | 9 | 4.69 |
| | | | *Trichosphaeriaceae* | *Nigrospora* | 15 | 7.81 |
| | | | *Didymellaceae* | *Epicoccum* | 8 | 4.17 |
| | | | *Davidiellaceae* | *Cladosporium* | 18 | 9.37 |
| Old | 50 | 300 | *Microascaceae* | *Aspergillus* | 159 | 53 |
| | | | *Trichocomaceae* | *Penicillium* | 25 | 8.33 |
| | | | *Nectriaceae* | *Fusarium* | 29 | 9.67 |
| | | | *Pleosporaceae* | *Alternaria* | 13 | 4.33 |
| | | | *Trichosphaeriaceae* | *Nigrospora* | 29 | 9.67 |
| | | | *Didymellaceae* | *Epicoccum* | 13 | 4.33 |
| | | | *Davidiellaceae* | *Cladosporium* | 32 | 10.67 |

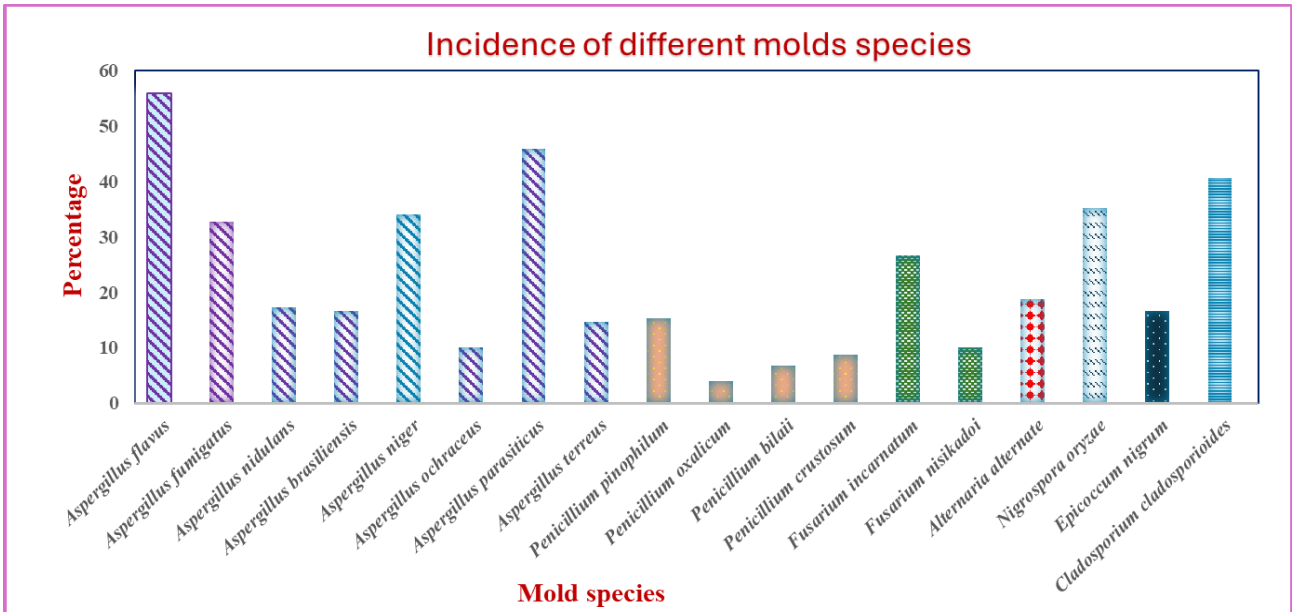


*Figure 1 Incidence of different molds species isolated from examined Ras cheese samples*

***Table 4 Incidence of Aspergillus flavus in different types of examined Ras cheese samples***

| Type of Ras cheese samples | Positive samples | |
|---|---|---|
| | No. | % |
| Recent | 22 | 44 |
| Medium | 25 | 50 |
| Old | 37 | 74 |
| Total/150 | 84 | 56 |

***Table 5 Occurrence and distribution of AFB1 in positive A. flavus Ras cheese samples by ELISA technique***

| Type of samples | No. of positive *A. flavus* samples | No. of positive *AFB1* samples | Positive samples | | |
|---|---|---|---|---|---|
| | | | Minimum (ng/kg) | Maximum (ng/kg) | Mean ± SE |
| Recent | 22 | 22 | 2.12 | 2.59 | 2.46 ± 0.03 |
| Medium | 25 | 25 | 0.87 | 2.64 | 2.37 ± 0.06 |
| Old | 37 | 37 | 0.74 | 2.62 | 2.44 ± 0.05 |

***Table 6 Comparing the detected levels of AFB1 (ng/kg) in examined Ras Cheese samples with EOSQC, EC regulation and US FDA***

| Type of samples | No. of positive *A. flavus* samples | Exceeding EOSQC (0 ng/kg) | | Exceeding EC regulation (0 ng/kg) | | Exceeding US FDA (0 ng/kg) | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Recent | 22 | 22 | 100 | 22 | 100 | 22 | 100 |
| Medium | 25 | 25 | 100 | 25 | 100 | 25 | 100 |
| Old | 37 | 37 | 100 | 37 | 100 | 37 | 100 |
| Total | 84 | 84 | 100 | 84 | 100 | 84 | 100 |

**EOSQC: Egyptian Organization for Standardization and Quality Control, (2010), *EC: European Commission regulation, (2010), US FDA: US FDA, (2011)***

***Table 7 Occurrence and distribution of AFM1 in positive A. flavus Ras cheese samples by ELISA technique***

| Type of samples | No. of positive *A. flavus* samples | No. of positive *AFM1* samples | Positive samples | | |
|---|---|---|---|---|---|
| | | | Minimum (ng/kg) | Maximum (ng/kg) | Mean ± SE |
| Recent | 22 | 22 | 9.1 | 236.2 | **79.7** ± 13.49 |
| Medium | 25 | 25 | 14.65 | 287.2 | **87.25** ± 14.71 |
| Old | 37 | 37 | 8.86 | 527.7 | **102.34** ± 17.24 |

***Table 8 Comparing the detected levels of AFM1 (ng/kg) in examined Ras Cheese samples with EOSQC, EC regulation and US FDA***

| Type of samples | No. of positive *A. flavus* samples | Exceeding EOSQC (50 ng/kg) | | Exceeding EC regulation (50 ng/kg) | | Exceeding US FDA (500 ng/kg) | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Recent | 22 | 13 | 59.1 | 13 | 59.1 | – | – |
| Medium | 25 | 14 | 56 | 14 | 56 | – | – |
| Old | 37 | 22 | 59.46 | 22 | 59.46 | 1 | 2.7 |
| Total | 84 | 49 | 58.3 | 49 | 58.3 | 1 | 1.19 |

***EOSQC: Egyptian Organization for Standardization and Quality Control, (2010), EC: European Commission regulation, (2010), US FDA: US FDA, (2011)***

## Discussion

The incidence of mold was 98, 100 and 100% with mean counts of $41\times10^5 \pm 14\times10^5$, $12\times10^6 \pm 1.9\times10^6$ and $48\times10^6 \pm 5.9\times10^6$ CFU/g in the examined recent, medium and old Ras cheese samples, respectively. Lower results were obtained by other studies [28,29,30,31] which mentioned that the mean count of mold in Ras cheese samples was $4.1x10^3 \pm 1.6x10^2$, $7.06 x10^4 \pm 3.27 x10^4$, $2.68 x10^4 \pm 0.51$ and $4.9 \times 10^5 \pm 0.83 \times 10^5$ CFU/g, respectively. It is obvious that the highest mean count was in the old Ras cheese examined samples. According to the Egyptian Organization for Standardization and Quality Control [32] which stipulated that Ras cheese should not contain more than 10 CFU/g of mold, it was evident that all examined recent, medium and old Ras cheese samples failed to comply with standards. Several factors can contribute to the contamination of various milk products by molds. These include unsanitary construction of milk processing plants, inadequate maintenance of pasteurization temperatures, the use of raw milk in cheese manufacturing, failure to adhere to good manufacturing practices, poor quality of raw materials, improper sterilization of equipment and utensils, omission of preservation methods, the use of unwholesome and non-potable water, and inadequate cleaning of packaging materials [33]. *Aspergillus* where the most prominent mold genus isolated from different Ras cheese samples, followed by genus *Cladosporium, Fusarium & Penicillium.* The isolated mold genus in this study agreed with those obtained by other researchers [34,28,35,4] who showed that *Aspergillus* was the most predominant isolated mold from Ras cheese. The incidence of *A. flavus* was higher in old Ras cheese samples with a percentage of 74% than in recent and medium Ras cheese samples with percentages of 44 and 50%, respectively; this may be related to the contamination from the surrounding environment and bad storage conditions. A lower incidence of *A. flavus* in Ras cheese samples was obtained by Zeinab et al. [30] who recorded an incidence of 2.78, while 21.15% incidence was detected by Seddek et al. [4]. All positive *A. flavus* samples in different types of Ras cheese samples were positive for AFB1. The presence of AFB1 in milk and milk products may resulted from the ingestion of feedstuffs containing AFB1 that the cow liver has not wholly metabolized to AFM1; therefore, AFB1 is found in milk, as well as the contamination of cheese with mold spores that produce AFB1 during processing and storage due to the lack or inadequate hygienic measures applied [36]. Similar results stated that all examined cheese samples were consistently positive for AFB1[37, 38]. In contrast, negative results were previously recorded [39,40]. Furthermore, higher AFB1 contamination levels were recorded at levels of an average 7.54 ± l.46 ng/kg [41]. All positive *A. flavus* samples in different types of Ras cheese samples were positive for AFM1. The concentration of AFM1 in milk depends on the amount of the ingested AFB1 by animals, where the liver metabolizes AFB1 into AFM1 and secreted in the milk. AFM1 is stable in raw milk and processed milk products which makes milk and dairy

products the primary vehicle for introducing AFM1 into the human diet [27]. AFM1 is hepatotoxic, mutagenic, and carcinogenic; its carcinogenicity is nearly 2-10% greater than AFB1 [15]. Our results of AFM1 were lower than those reported by other researchers [42,43,44] where the mean values were 132.24, 4800 and 4900 ng/kg, respectively. While it was higher than other studies [45,46,47,48,27] which reported that the examined Ras cheese samples were contaminated with AFM1 with a mean of 21 ± 5, 22.93 ± 6.31, 15± 1, 56.048 ± 6.29 and 51.05± 6.19 ng/kg, respectively. Due to the hazardous nature of AFM1 along with its extreme thermal resistance, most countries established legal regulations for AFM1 in raw milk and dairy products with an admissible limit, which varies from 50 ng/ kg recommended by the Egyptian standard [17] and EC regulation [18] to 500 ng/kg established by the Codex Alimentarius Commission and US Food and Drug Administration [19].

## Conclusion

From this study, it is clear that the production of Ras cheese involves several risks of contamination due to the lack of pasteurization, poor quality and unhygienic handling and distribution methods. Two months (the least time for Ras cheese maturation) is enough for cheese contamination with molds and their aflatoxins.

## Authors' contribution

The work was equally distributed between authors. All authors have read and approved the final version of the manuscript.

## Conflict of interest

There is no conflict of interest.

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## References

1. Eldenary AM. Hazard Analysis of Ras Cheese in Egyptian Delta Governorates. Journal of Sustainable Agricultural and Environmental Sciences. 2023 Jan 1;2(1):130-56.

2. Hattem HE, Taleb AT, Manal AN, Hanaa SS. Effect of pasteurization and season on milk composition and ripening of Ras cheese. Journal of Brewing and Distilling. 2012 Mar;3(2):15-22.

3. Hammam AR, Elfaruk MS, Ahmed ME, Sunkesula V. Characteristics and technological aspects of the Egyptian cheeses. International Journal of Current Microbiology and Applied Sciences. 2020 Jun;9(6):3338-54.

4. Seddek NH, Gomah NH, Osman DM. Fungal flora contaminating Egyptian Ras cheese with reference to their toxins and enzymes. Food Science and Technology. 2016;4(4):64-8.

5. Kure CF, Skaar I. The fungal problem in cheese industry. Current Opinion in Food Science. 2019 Oct 1;29: 14-9.

6. Awad S. Texture and flavour development in Ras cheese made from raw and pasteurized milk. Food chemistry. 2006 Aug 1;97(3):394-400.

7. Taniwaki MH. Editorial overview: Food mycology. Current Opinion in Food Science. 2018 Oct 1;23:vi-i.

8. Garnier L, Valence F, Mounier J. Diversity and control of spoilage fungi in dairy products: An update. Microorganisms. 2017 Jul 28;5(3):42.

9. Anelli P, Haidukowski M, Epifani F, Cimmarusti MT, Moretti A, Logrieco A, Susca A. Fungal mycobiota and mycotoxin risk for traditional artisan Italian cave cheese. Food Microbiology. 2019 Apr 1;78: 62-72.

10. Kumeda Y, Asao T. Heteroduplex panel analysis, a novel method for genetic identification of *Aspergillus* section *Flavi* strains. Applied and Environmental Microbiology. 2001 Sep 1;67(9):4084-90.

11. Rodrigues P, Soares C, Kozakiewicz Z, Paterson R, Lima N, Venâncio A. Identification and characterization of *Aspergillus flavus* and aflatoxins. Applied Microbiology. 2007: 527-34.

12. Prandini A, Tansini GI, Sigolo S, Filippi LA, Laporta M, Piva G. On the occurrence of aflatoxin M1 in milk and dairy products. Food and chemical toxicology. 2009 May 1;47(5):984-91.

13. Giray B, Girgin G, Engin AB, Aydın S, Sahin G. Aflatoxin levels in wheat samples consumed in some regions of Turkey. Food control. 2007 Jan 1;18(1):23-9.

14. Hussain I, Anwar J, Munawar MA, Asi MR. Variation of levels of aflatoxin M1 in raw milk from different localities in the central areas of Punjab, Pakistan. Food Control. 2008 Dec 1;19(12):1126-9.

15. International Agency for Research on Cancer. Traditional Herbal Medicines, Some mycotoxins, naphtalene and styrene. IARC monographs on the evaluation of carcinogenic risks to humans. IARC Scientific Publications. 2002:171-300.

16. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. IARC Scientific Publications. 2012:100-385.

17. EOSQC: Egyptian Organization for Standardization and Quality Control. Egyptian Standards (E.S.) number

7136/2010: Maximum levels of mycotoxin for foods and feeds. Part-1: Aflatoxins number 1875.2010.

18. EC regulation: European Commission Regulation. No. 165, Amending Regulation (EC). Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Aflatoxins. Official Journal of the European Union. 2010: 8-12.

19. US FDA. Guidance for industry: Action levels for poisonous or deleterious substances in human food and animal feed. Food and Drug Administration; (April 2011) 20/04/2011. Available from: http:// www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/ Guidance Documents/Chemical Contaminants and Pesticides/ ucm077969.htm.

20. APHA ''American Public Health Association'': Compendium of methods for the microbiological examination of foods. 4thEd. Eds. Downes P. and K. Ito. Sheridan Books Inc.,Washington D.C., USA; 2001.

21. Domsch KH, Games W, Anderson T: Compendium of soil fungi. 2nd ed. IHW Verlag. Eching, Germany. 2007; 627 pages.

22. Pitt JI. The genus *Penicillium* and its teleomorphic states Eupenicillium and Talaromyces. Academic Press Inc. Ltd. 1979; London, pp 635.

23. Raper KB, Fennell DI. The genus *Aspergillus*., Williams and Wilkins Company, Baltimore. 1965; pp 686.

24. Leslie JF, Summerell BA. The *Fusarium* laboratory manual. Blackwell, Oxford.2006; pp388.

25. Ismail MA, Abdel-Hafez SI, Hussein NA, Abdel-Hameed NA. Contributions to the genus *Fusarium* in Egypt with dichotomous keys for identification of species. Suchy Las, Poland. Publisher Tomasz M. Karpiński. 2015; 175.

26. Simmons EG. *Alternaria* an identification manual, fully illustrated and with catalogue raisonné 1796-2007. CBS Fungal Biodiversity Centre, Utrecht, The Netherlands. 2007.

27. Esam RM, Hafez RS, Khafaga NI, Fahim KM, Ahmed LI. Assessment of aflatoxin M1 and B1 in some dairy products with referring to the analytical performances of enzyme-linked immunosorbent assay in comparison to high-performance liquid chromatography. Veterinary World. 2022 Jan;15(1):91.

28. EL-bagory AM, Eid AM, Hammad AM, Dawood SA. Prevalence of fungi in locally produced cheese and molecular characterization of isolated toxigenic molds. Benha Veterinary Medical Journal. 2014 Dec;27(2):9-20.

29. El-Leboudy AA, Amer AA, Youssef MR. Assessment of sanitary measures of Ras cheese in manufacturing dairy plant in Alexandria Governorate. Alexandria Journal of Veterinary Sciences. 2014; 40:87-94

30. Zeinab AM, Abbas AA, Amr AA, Youssef SY. Incidence of mycobiota in some dairy products and its public health hazards. Alexandria Journal for Veterinary Sciences. 2017; 53(1):203-10.

31. Elramly MH, El-Leboudy AA, Al-Ansary MA. Mycological evaluation of Egyptian Ras cheese with special reference to mycotoxins. Alexandria Journal for Veterinary Sciences. 2019; 63(2):33-8.

32. Egyptian Standards (E.S.). Hard cheese - part 5 - Ras cheese. Egyptian Organization for Standardization. 1007-5/2005.

33. Pitt, J.I. and Hocking, A.D. Fungi and food spoilage. Second ed. Gaithersburg: Aspen Publishers. 1999.

34. Cheong EY, Sandhu A, Jayabalan J, Le TT, Nhiep NT, Ho HT, Zwielehner J, Bansal N, Turner MS. Isolation of lactic acid bacteria with antifungal activity against the common cheese spoilage mold *Penicillium* commune and their potential as biopreservatives in cheese. Food Control. 2014 Dec 1; 46:91-7.

35. El-Fadaly HM, El-Kadi SM, Hamad MN, Habib AA. Isolation and identification of Egyptian Ras cheese (Romy) contaminating fungi during ripening period. Journal of Microbiology Research. 2015; 5(1):1-0.

36. Duarte SC, Almeida AM, Teixeira AS, Pereira AL, Falcão AC, Pena A, Lino CM. Aflatoxin M1 in marketed milk in Portugal: Assessment of human and animal exposure. Food control. 2013 Apr 1;30(2): 411-7.

37. Abou Ayana IA, El-Deen AG, Ayyad KM, El-Metwally MA. Influence of some Abiotic Anti-Fungi and Saccharomyces cerevisiae on Aflatoxin B1 and Isolated Fungi from some Cheese Types. International Journal of Dairy Science. 2014;9(2): 32-44.

38. Mao J, Zheng N, Wen F, Guo L, Fu C, Ouyang H, Zhong L, Wang J, Lei S. Multi-mycotoxins analysis in raw milk by ultra high performance liquid chromatography coupled to quadrupole orbitrap mass spectrometry. Food Control. 2018 Feb 1;84: 305-11.

39. Montagna MT, Napoli C, De Giglio O, Iatta R, Barbuti G. Occurrence of aflatoxin M1 in dairy products in southern Italy. International Journal of Molecular Sciences. 2008 Dec 12;9(12): 2614-21.

40. Embaby EM, Awni NM, Abdel-Galil MM, El-Gendy HI. Distribution of fungi and mycotoxins associated some foods. Middle East Journal of Applied Sciences. 2015;5(3):734-41.

41. Abd Elkhalek A. Incidence of aflatoxins B1 and M1 in raw milk and some cheeses. Mansoura Veterinary Medical Journal. 2001 Jun 1;3(1):11-23.

42. Amer AA, Ibrahim ME. Determination of aflatoxin M1 in raw milk and traditional cheeses retailed in Egyptian markets. Journal of Toxicology and Environmental Health Sciences. 2010 Sep;2(4): 50-3.

43. Nassib TA, Guergues SN, Motawee MM. Comparison between some different methods for determination of Aflatoxin M1 in milk and some dairy products. The Egyptian Journal of Hospital Medicine. 2005 Jan 1;18(1): 43-7.

44. Abdel-All SM, Abd-El-Ghany MA, Motawee MM. Inhibition of *Aspergillus* growth and aflatoxins production in some dairy products. InThe 3ed annual conference of quality education development in Egypt and the Arab region to achievement the requirements of job markets in the globalization age (strategic vision). Faculty of Specific Education Mansoura University. 2008 Apr; pp. 1108-1120

45. Hosny G, El-Sadany MA, Atwa MA. Monitoring of aflatoxin M1 in some dairy products in local market of Alexandria, Egypt: Attempts for detoxification. Journal of Food and Dairy Sciences. 2014 Feb 1;5(2):103-24.

46. Ahmed KM, Hafez RS, Morgan SD, Awad AA. Detection of some chemical hazards in milk and some dairy products. African Journal of Food Science. 2015 Apr 30;9(4):187-93.

47. Younis G, Ibrahim D, Awad A, El Bardisy MM. Determination of aflatoxin M1 and ochratoxin A in milk and dairy products in supermarkets located in Mansoura City, Egypt. Advances in Animal and Veterinary Sciences. 2016;4(2):114-21.

48. Aiad AS and Abo El-makarem HS. Aflatoxin M1 levels in milk and some dairy products in Alexandria city. Assiut Veterinary Medical Journal. 2013 Nov 6;59(139): 93-8.