15 *Egypt. J. Vet. Sci.* **Vol. 56, (Special issue),** pp. 141-153 (2025)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Diversity of Toxigenic Molds and Aflatoxins in Different Varieties of Dairy Products and its Substitutes

Adam A.H.*[1], Salwa A. Aly[1], Saad M.F.[1], Rasha M.H. Sayed-ElAhl[2] and Aml S. Ibrahim[1]

[1] *Department of Food Hygiene & Control, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.*

[2] *Department of Mycology and Mycotoxins, Animal Health Research Institute (AHRI), Agriculture Research Centre (ARC), Giza, Egypt.*

Abstract

ALTHOUGH dairy products are more exposed to mold growth, dairy substitutes are also at risk. The mold contamination issue in dairy products extends beyond quality concerns, encompassing broader food safety and public health apprehensions. In this regard, investigation of mold occurrence, identification of prevailing molds, and monitoring of aflatoxins levels via ELISA were accomplished on 210 samples of different dairy products and dairy substitutes (30 of each), that were obtained from various markets in El-Fayoum province, Egypt. Molds' highest contamination level was found in Ras cheese (73.3%) with the lowest compliance to the Egyptian Standards (ES), moreover, the lowest one (33.3%) and highest acceptability was found in cream analogue samples. There was a significant difference (p<0.05) between the mean values of Ras cheese and cheese analogue samples, alongside the means of plain yogurt and flavored yogurt samples. Aspergillus spp. was the most isolated mold (54.5%), followed by other species such as Rhizopus (22.8%), Penicillium (13.1%), Mucor (4.8%), Alternaria (3.4%), and Ulocladium (1.4%). *Aspergillus flavus* characterization by PCR revealed that 100.0% of the isolates were confirmed, while 85.3% harbored the aflatoxin B_1 aflR gene. Aflatoxin M_1 was detected in all the analyzed Ras cheese, 70.0% plain yogurt, 56.7% flavored yogurt, 63.3% milk powder, and 46.7% milk-based cereals samples, with mean values of 89.05±6.21, 63.42±5.04, 48.71±2.65, 51.70±2.55, and 35.22±3.36 ng/kg, respectively. Total aflatoxins were measured in cheese analogue and cream analogue samples with mean levels of 12.32±1.58 and 7.46±0.45 ppb, respectively. A great portion of the evaluated samples contained not only varying levels of Aspergillus spp. contamination, but also their aflatoxins. This necessitates the frequent monitoring of total aflatoxins in dairy substitutes and AFM_1 in dairy products. Moreover, applying strict control measures during various stages of production and processing of such products, as well as the proper handling and storage of animal feed, would reduce mold growth.

Keywords: Aflatoxin M_1, *Aspergillus flavus*, Dairy substitutes, ELISA, Milk-based cereals, Total aflatoxins.

Introduction

Dairy products are a vital component of the human diet, especially for infants and young children, as they are a rich source of essential nutrients, including calcium, protein, and vitamins. However, their inherent nutrient richness and moisture content create an ideal environment for various microorganisms to thrive, including molds [1]. There is an increasing global demand for dairy substitutes, which constitutes the need for commercial-scale production. This demand is driven due to its unique flavor and low price [2].

Mold contamination in milk products is of particular concern in the food business. It mainly occurs due to post-processing contaminations or heat-resistance mold spores [3]. The growth of molds in dairy products is influenced by various parameters, including temperature, humidity, pH, and the composition of the product. Although molds are not foodborne pathogens by themselves, they can produce secondary toxic metabolites known as mycotoxins [4].

Aflatoxins are secondary toxic metabolites produced by certain molds, primarily *Aspergillus flavus* and *Aspergillus parasiticus*. These compounds pose a significant risk to public health due to their carcinogenic, mutagenic, and immunosuppressive properties. This is largely attributed to the metabolic conversion of ingested aflatoxins in livestock feed

*Corresponding authors: Adam A.H, E-mail: abdulkareem.adam@cu.edu.eg, Tel.: 01004726462
(Received 13 March 2025, accepted 18 April 2025)
DOI: 10.21608/ejvs.2025.368109.2699

into aflatoxin M_1 (AFM_1), a hydroxylated metabolite that is excreted into milk and consequently incorporated into dairy products [5, 6]. The conversion factor of AFB_1 from animal feed to AFM_1 in raw milk is 0.30-6.20%, depending on genetics, feed received, and lactation stage [7].

When dairy products are manufactured from contaminated milk with AFM_1, the toxin remains heat-resistant, allowing it to persist in processed dairy products such as cheese and yogurt [8]. Dairy substitutes like plant-based cheese are typically not directly impacted by aflatoxins since they nearly do not involve animal milk, though contamination may occur if raw ingredients used in processing are contaminated [9]. Therefore, the existence of aflatoxins in dairy products and their substitutes is particularly alarming owing to their widespread consumption across age groups, especially among children who are highly vulnerable to its adverse effects.

The International Agency for Research on Cancer (IARC) has classified aflatoxins (AFB_1 and AFM_1) as carcinogenic to humans. While AFM_1 exhibits lower mutagenic and carcinogenic potency compared to AFB_1, the carcinogenic effects of AFM_1 are still significant enough to place it in Group 1 of human carcinogens [10, 11]. In developed countries, the risk posed by aflatoxins has prompted the implementation of stringent regulatory limits in milk and dairy products [12]. Several countries have set up maximum acceptable limits of aflatoxins in food that are expected to be safe. European Commission [13] set at 4-15 ppb, while the United States Food and Drug Administration (US FDA) [14] at 20 ppb. Regarding AFM_1 in milk and its products, limits vary from 50 ng/kg established by Egyptian Standards [15] and European Commission [13] to 500 ng/kg set by US FDA. However, research highlights that these limits are not always met, particularly in regions with poor storage conditions or inadequate feed monitoring [9, 16]. Therefore, this study aimed to monitor distribution of molds, identify and detect toxigenic strains producing aflatoxins by DNA-based detection methods (PCR), in addition, quantitative assessment of aflatoxin M_1 and total aflatoxins in most popular milk products and their substitutes consumed in Egypt.

Materials and Methods

Samples collection

A total of two hundred and ten samples of Ras cheese, plain yogurt, flavored yogurt, milk powder, milk-based cereals, cheese analogues (soft cheese with vegetable fats), and cream analogues (30 samples each) were analyzed. These samples were purchased from various marketplaces and retail establishments in El-Fayoum governorate, Egypt; while milk-based cereals samples were collected from different pharmacies. The quantity of each sample ranged from 250-300 grams. The gathered samples were promptly transferred to the laboratory with the least amount of delay to minimize any potential contamination.

Samples preparation

Eleven grams of each sample were aseptically transferred into a sterile blending container. To this, 99 ml of 1% peptone water (Himedia, RM001) or 2% sodium citrate for cheese samples (Himedia, GRM255) were added, and the mixture was homogenized using a sterile blending device (Lab-Blender/400-Seward, Worthing UK) for a duration of 2 minutes. Subsequently, serial dilutions of the homogenized mixture were prepared in a tenfold manner [17].

Mold isolation

Duplicate plates of sterile Sabouraud’s dextrose agar (SDA) (Himedia, MH063) supplemented with 0.05 mg/ml chloramphenicol (Himedia, CMS218) were used for mold isolation by inoculating various dilutions using the pouring technique. The inoculated plates were then placed in an incubator set at 25°C and left to incubate for a period of 5 days [18].

Identification of the isolated mold

Macroscopic and microscopic identification:

The isolates were recognized using macroscopic and microscopic means. Macroscopic observations were conducted to assess the color, texture, size, edges, surface, and reverse pigmentation of the colonies on Malt extract agar (MEA) (Himedia, M1913) and Czapek’s Yeast extract agar (CYA) (Himedia, M1335). For microscopic examination, a lactophenol cotton blue stain (Himedia, S016) was utilized. A drop of the stain was carefully placed on a clean slide free from grease or any contaminants. Subsequently, the material was gently teased apart, and a cover slip was placed over the sample to facilitate microscopic observation [19, 20].

Electron microscopic identification:

Electron microscopy examination was conducted to visualize the ultrastructure of the mold isolates at the microscopic level. By employing electron beams instead of light. Cultured cells were fixed and washed followed by post-fixation washing and dehydration. Subsequently, it is situated on stub and covered with a tiny layer of a conductive substance using the sputter coating technique [21, 22].

DNA extraction and polymerase chain reaction (PCR)

Extraction of Deoxyribonucleic acid from the identified *A. flavus* isolates was carried out using a Gene JET purification kit (Thermo Fisher, K0721). PCR Master Mix: DreamTaq Green PCR Master Mix (2X) Fermentas Company, Cat. No. K1080, USA was employed. The Aflatoxin biosynthesis gene cluster (accession no. AY510455) were amplified using the polymerase chain reaction (PCR) technique for identification of *A. flavus*, and while the Aflatoxin B_1 regulatory gene fragments (aflR) (accession no. KY769957) was amplified to determine *A. flavus* toxigenicity. The specific sequence of aflatoxin biosynthesis gene cluster primers was (F: 5′ GGTGGTGAAGAAGTCTATCTAAGG 3′) and (R: 5′ AAGGCATAAAGGGTGTGGAG 3′), and the specific sequence of aflR primers was (F: 5′ AACCGCATCCACAATCTCAT 3′) and (R: 5′ :AGTGCAGTTCGCTCAGAACA 3′) [23, 24].

Quantitative determination of AFM_1 and total aflatoxins using ELISA assay

The quantitative analysis of AFM_1 levels in the samples was carried out using a competitive enzyme-linked immunosorbent assay based on the RIDASCREEN® Aflatoxin M_1 (Art. no.: R1121, r-biopharm, Darmstadt, Germany) test kit. Most of the used reagents were provided by the kit manufacturer. The ELISA test procedure was conducted using the instructions of RIDASCREEN® test kit according to ISO (14675:2003) [25], the limit of detection (LOD) was 5 ng/kg. For total aflatoxins measurement the MaxSignal® Total Aflatoxin (B_1, B_2, G_1, and G_2) ELISA Kit was implemented following the manufacturer's instructions.

Recovery study

For the validation of the method, a recovery study was achieved by spiking known concentrations (5–80 ng/kg) of AFM_1 according to the instructions for use of the RIDASCREEN kit. The mean recovery score was 99.00%, with a coefficient of variation 1.4% (Fig. 1).

Statistical analysis

The one-way ANOVA and t-test were used to examine the outcomes of various parameters through SPSS version 25. The findings mean values were expected to be significantly different if the *p*-value was less than 0.05.

Results

Mold count, isolation, and identification

The data illustrated in Table (1) showed that the highest level of mold contamination was found in Ras cheese (73.3%), flavored yogurt (56.6%), and cheese analogue (50.0%) samples with mean counts of $12×10^4 ± 7.5×10^4$, $49×10^3 ± 18×10^3$, and $2.9×10^2 ± 2.3×10^2$ CFU/g, respectively. Plain yogurt samples were contaminated in a percentage of (46.7%), milk-based cereals (46.7%), and milk powder (40.0%) with average counts of $27×10^3 ± 9.6×10^3$, $42×10 ± 13×10$, and $17×10^2 ± 3.6×10^2$ CFU/g, respectively. Meanwhile, the lowest contamination level was found in cream analogue samples (33.3%) with an average value of $4.5×10 ± 1.1×10$ CFU/g. Based on the statistical outcomes of the analyzed samples, there was a significant difference ($p<0.05$) between the means of Ras cheese and cheese analogue samples. In addition, average values of plain yogurt and flavored yogurt samples showed significant differences.

The highest frequency was distributed between 10^3 to $<10^4$ CFU/g for the examined Ras cheese and flavored yogurt samples. In case of counts between 10^2 to $<10^3$ CFU/g, plain yogurt, milk-based cereals, and cream analogue samples values were the most prominent (Table 2).

Furthermore, compliance with the Egyptian Standards (ES) regarding the mold count, the highest percentages of acceptability (76.7%) was detected in cream analogue samples followed by milk powder samples (60.0%). Ras cheese samples had the lowest acceptability percentage (26.7%), as displayed in (Fig. 2).

Aspergillus spp. was the most isolated mold with 54.5%, followed by other species such as Rhizopus, Penicillium, Mucor, Alternaria, and Ulocladium with 22.8%, 13.1%, 4.8%, 3.4%, and 1.4%, respectively. *Aspergillus flavus* possessed the greatest percentage among the isolated species predominated in Ras cheese, plain yogurt, and cheese analogue samples in a percentage of 28.6%, 33.3%, and 38.9%, respectively (Table 3).

Thirty-four strains of *Aspergillus flavus* subjected to macroscopic, microscopic, electron microscopy as well as PCR identification techniques. *Aspergillus flavus aflatoxin biosynthesis gene cluster* was detected in 100.0% of the tested strains (Fig. 3 and Fig. 4). Toxin production capability was confirmed relying on the detection of *Aflatoxin B_1 (aflR)* gene that was detected in 85.3% of the tested strains (Table 4 and Figure 5).

Incidence of aflatoxin M_1 and total aflatoxins

The Aflatoxin M_1 prevalence in the investigated Ras cheese, plain yogurt, flavored yogurt, milk powder, and milk-based cereals samples was summarized in Table 5. All the examined Ras cheese samples (100.0%) contained AFM_1, with concentrations ranging from 37.3 to 193.6 ng/kg and a mean value of 89.05 ± 6.21 ng/kg. Regarding the contaminated yogurt samples, AFM_1 mean concentrations were 63.42 ± 5.04 and 48.71 ± 2.65 ng/kg in plain and flavored yogurt samples, respectively. The average values of AFM_1 in milk powder and milk-based cereals samples were 51.70 ± 2.55 and 35.22 ± 3.36 ng/kg, respectively. Out of the Ras cheese samples that were evaluated, 80.0% of them exceeded EC regulations and Egyptian Standards limits (<50 ng/kg), whereas 52.4% and 35.3% of the assessed plain and flavored yogurt samples exceeded those limits. Milk powder and milk-based cereals samples showed a nearly similar percentage of unacceptability (42.1% and 42.9%) for those standards. None of the evaluated Ras cheese, plain yogurt, flavored yogurt, milk powder, or milk-based cereals samples exceeded the US Food and Drug Administration limit (<500 ng/kg). Regarding the findings exhibited in Table (6), the highest frequency distribution of AFM_1 in Ras cheese (56.7%) and plain yogurt samples (20%) lies within the range of 81-120 ng/kg, respectively.

The data illustrated in Table (7) shows that 46.6% of cheese analogue samples contained total aflatoxins in a concentration range of 7.41 - 25.13 ppb with a mean value of 12.32 ± 1.58 ppb. Alternatively, 30.0% of cream analogue samples had an average value of contamination (7.46 ± 0.45 ppb) in a concentration range of 6.23 - 11.21 ppb. Out of the positive cheese analogue samples containing total aflatoxins, a percentage of 57.1% was unacceptable to the total aflatoxins standard limits stipulated by the EC (4-15 ppb for food) [13] and the US regulations (20 ppb for food) [14].

Discussion

Mold count, isolation, and identification

Molds commonly contaminate milk products due to their favorable growth conditions [1]. Our results of Ras cheese samples were higher than those reported by Ahmed et al. [26], who detected mold in Ras cheese with a mean count of 3.54×10^3 CFU/g.

The tested plain yogurt samples by Worku et al. [27] showed a coincident finding with this study, where the average mold count was 1.0×10^4 CFU/g. A greater contamination level (8.0×10^6 CFU/g) than our samples was documented by Keta et al. [28]. Concerning flavored yogurt samples, the obtained results were greater than those reported by Aman et al. [29], who detected mold with a mean value of 4.25×10^2 CFU/g. Recently, the growing use of dried milk has made its microbial quality a primary concern due to the high susceptibility of consumers to food-borne diseases [30]. In the current study, mold count findings of milk powder samples were in line with those reported by Abdelkhalek et al. [31], whose tested samples' mean value was 1.3×10^2 CFU/g. Abdel-Hameed et al. [32] revealed lower results, where mold was detected in an average value of 5.6×10 CFU/g. Moreover, Ibrahim et al. [33] declared that all the investigated milk powder samples had a mold level of less than 10 CFU/g. The milk-based cereals samples analyzed by Aidoo et al. [34] showed comparable mean values (6.31×10^2 CFU/g) to our mold count findings, meanwhile Ajayi and Okiti [35] recorded a higher mold value of 4.1×10^5 CFU/g in the examined milk-based cereals. The high mold counts in the surveyed products may be due to inadequate hygiene during processing, contamination after heat treatment, the heat resistance of mold spores, or the usage of inferior flavoring substances [36, 37].

Mycological analysis of cheese analogue samples mentioned by Hamad et al. [38] showed contamination in accordance with our findings, with a mean value of 3.98×10^2 CFU/g. Conversely, Mohamed et al. [39] reported a lower result, where cheese analogue samples contained mold in a mean count of 1.6×10 CFU/g. Our cream analogue samples contained mold count lower than those of El-gendi and El-shreef [40], where mold was detected in 66.7% of cream analogue samples with average count of $1.41\text{x}10^3$ CFU/g. The reduced mold count in dairy substitutes is likely due to the usage of various non-dairy fats and/or proteins in their production, along with the effective use of preservatives like potassium sorbate in these products' manufacturing [41].

The isolated mold strains in this study aligned with findings from Seddek et al. [42], who identified Aspergillus as the most common mold in Ras cheese, with *A. flavus* and *A. niger* being the most prevalent. On matching our results with those obtained by Fetouh et al. [43], *Aspergillus flavus*, *Aspergillus niger*, Penicillium spp., Rhizopus spp., and Mucor spp. could be isolated from yogurt samples. Oyeyipo et al. [44] found that Aspergillus was the most predominant in powdered milk samples, followed by Mucor, Penicillium, and Rhizopus. Aidoo et al. [34] reported that the genera Aspergillus, Penicillium, and Mucor were isolated from the analyzed milk-based cereals samples. Salim et al. [45] identified Aspergillus as the most detected mold in cheese analogue samples before Penicillium, which is in accordance with our findings. Further studies are needed on identifying mold species in cream analogues as the currently available data is insufficient.

This study aimed to develop a specific alternative to traditional aflatoxin quantification methods. PCR was used to detect the aflatoxin B_1 regulatory gene (*aflR*), indicating whether isolated *A. flavus* strains can produce AFB_1 in contaminated products. The PCR technique enables the screening of numerous suspected samples with great accuracy and sensitivity, allowing for the rapid processing of large numbers of samples [46]. Conventionally identified *A. flavus* strains that were subjected to genomic analysis contained these targeted gene fragments, agreed with the PCR results reported by Salim et al. [45]. The ability to synthesize AFB_1 was detected in 85.3% of *A. flavus* isolates from the analyzed products, reflecting the high risk of contaminated dairy and non-dairy products by *A. flavus*.

Incidence of aflatoxin M_1 and total aflatoxins

Mold counts exceeding permissible limits not only indicate compromised product quality but also raise concerns regarding potential mycotoxin contamination. The aforementioned results linked to Ras cheese were agreeable with Amer and Ibrahim [47], who detected AFM_1 in 100.0% of the tested Ras cheese samples, ranging from 51.6 to 182 ng/kg. We found lower values than those documented by Ahmed et al. [26], AFM_1 mean value was 890 ng/kg and all the samples exceeded the acceptable levels declared by ES and EC regulations.

Aflatoxin M_1 mean content in plain yogurt samples correlate fairly with Camaj Ibrahimi et al. [48] findings. A lower AFM_1 concentration was found with respect to those reported by Corassin et al. [49], who detected AFM_1 in mean value of 50 ng/kg. Aflatoxin M_1 exceeded the EC regulations in 40% of yogurt samples analyzed by Camaj Ibrahimi et al. [48]. Parallel to the current study the examined flavored yogurt samples were loaded with AFM_1 in a mean value of 45.3 ng/kg [50]. We found much higher values for AFM_1 than Heshmati et al. [51], AFM_1 mean value was 29 ng/kg. The detection of AFM_1 in the examined yogurt samples highlighted the significance of fermented dairy products as sources of aflatoxins to consumers.

Milk powder results differ to some extent with Alahlah et al. [52], who detected AFM_1 in a lower value (25 ng/kg). On the contrary, the findings were lower than those observed by Marimón Sibaja et al. [53], who reported AFM_1 concentration of 600 ng/kg. The current study findings of AFM_1 in powdered milk were nearly coincided with those recorded by Iqbal et al. [11], AFM_1 mean value was 57.3 ng/kg. Residues of AFM_1 were detected in milk powder samples at higher concentrations than the maximum permitted levels by the Egyptian and European regulations [54].

Aflatoxin M_1 mean level of milk-based cereals samples was lower than those documented by Marimón Sibaja et al. [53], AFM_1 mean concentration was 250 ng/kg. While the investigated samples by Elaridi et al. [55] had similar levels of contamination (33.7 ng/kg) as observed in the present study. However, comparatively lower levels of AFM_1 in milk-based cereals (9.38 ng/kg) was detected by Malissiova et al. [56]. The variation in detected AFM_1 concentrations in forgoing studies may be related to different factors, including differences in feeding systems, farm management practices, changes in seasons' climates, analytical methods, product preparation, storage, and packaging [57].

Previous studies showed that AFM_1 binds strongly to milk casein, resulting in cheese containing four times more AFM_1 than milk. Therefore, cheese may be the highest source of aflatoxins among dairy products [58]. AFM_1 remains bound to casein throughout dairy production processes, including those for powdered milk, cheese, and yogurt. Due to its strong binding and high heat resistance, AFM_1 cannot be eliminated by conventional heat treatments [59]. Remarkably, breast feeding, milk-based cereals and infant formulae are the main sources for infant feeding; consequently, the presence of toxic substances, such as AFM_1, in these food categories may contribute to a greater exposure comparable to adults [60].

Assessing dairy substitutes by determining the total aflatoxins may be valuable and preferable, which is a more practical way to assess the crucial health risk associated with aflatoxins producing mold in these products. Embaby et al. [61] reported a higher result of total aflatoxins (298 ppb) in cheese analogue samples. Aflatoxin contamination can arise either from contaminated additives (dried fruit, spices, and oil seeds), or accidental contamination of raw materials [62]. The lack of research on mold and toxins in dairy substitutes makes it challenging to assess their impact on public health and food safety. This gap underscores the need for further studies to understand potential risks and establish safety standards.

Conclusion

A broad mold diversity and aflatoxins mixture were found in the locally manufactured dairy products and dairy substitutes, which denotes the unhygienic conditions of either processing or keeping in dairy shops. Aspergillus and Penicillium species were the most predominant isolated mold from the examined products. The results showed a high percentage of the examined dairy products samples' that contrasted with Egyptian Standards, leading to high health risks for consumers. So, it is recommended to adopt strict control measures during various stages of production and processing of such products. Conducting regulatory and monitoring measures for controlling aflatoxins production and inhibition of toxigenic mold growth in food and feed are essential to mitigate the higher levels of AFB_1 and AFM_1 in these products. Protecting dairy animals from eating contaminated feed with AFB_1 is the best way to prevent the generation of AFM_1 in dairy products. Efforts to prevent and control AFM_1 in milk and its products should be addressed.

Acknowledgments

The authors would like to thank all the participants for helping to finish this study.

Funding statement

No funding was obtained for this study.

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest regarding the research data and tool.

TABLE 1. Statistical analysis results of the total mold count (CFU/g) in the tested samples (n=210)

Type of samples	Mold count				
	Positive samples		Values		
	No.	%	Min	Max	Mean ± S.E.M
Ras cheese	22	73.3	<10	13×10^{5}	$1.2\times10^{5} \pm 7.5\times10^{4a}$
Plain yogurt	14	46.7	<10	10×10^{4}	$2.7\times10^{4} \pm 9.6\times10^{3b}$
Flavored yogurt	17	56.7	<10	30×10^{4}	$4.9\times10^{4} \pm 1.8\times10^{4b}$
Milk powder	12	40.0	<10	60×10^{2}	$1.7\times10^{3} \pm 3.6\times10^{2}$
Milk-based cereals	14	46.7	<10	57×10^{2}	$4.2\times10^{2} \pm 9.0\times10$
Cheese analogue	15	50.0	<10	23×10^{3}	$2.9\times10^{2} \pm 1.3\times10^{2a}$
Cream analogue	10	33.3	<10	12×10	$4.5\times10 \pm 1.1\times10$

n: number of the examined samples Min: minimum, Max: maximum SEM: standard error of mean
[a] There is a significant difference between the mean values of Ras cheese and cheese analogue samples ($p<0.05$).
[b] There is a significant difference between the mean values of plain yogurt and flavored yogurt samples ($p<0.05$).

TABLE 2. Frequency distribution of total mold count in the examined samples (n=210)

Sample type	Intervals				
	$10 - <10^{2}$	$10^{2} - <10^{3}$	$10^{3} - <10^{4}$	$10^{4} - <10^{5}$	$10^{5} - <10^{6}$
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Ras cheese	0 (0.0)	7 (23.3)	8 (26.7)	5 (16.7)	2 (6.6)
Plain yogurt	1 (3.3)	5 (16.7)	3 (10.0)	4 (13.3)	1 (3.3)
Flavored yogurt	1 (3.3)	4 (13.3)	7 (23.3)	2 (6.6)	3 (10.0)
Milk powder	2 (6.6)	5 (16.7)	5 (16.7)	0 (0.0)	0 (0.0)
Milk-based cereals	3 (10.0)	9 (30.0)	2 (6.7)	0 (0.0)	0 (0.0)
Cheese analogue	8 (26.7)	2 (6.6)	4 (13.3)	1 (3.3)	0 (0.0)
Cream analogue	3 (10.0)	5 (16.7)	2 (6.6)	0 (0.0)	0 (0.0)

n: number of the examined samples No.: number of the positive samples
Percentages were calculated in relation to the number of each examined product (n=30)

TABLE 3. Incidence of the isolated mold species in the examined samples (N=145)

Identified isolates		Ras cheese	Plain yogurt	Flavored yogurt	Milk powder	Milk-based cereals	Cheese analogue	Cream analogue
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Aspergillus spp.	79 (54.5)	20 (57.1)	12 (57.1)	10 (43.5)	7 (43.8)	12 (75.0)	12 (66.7)	6 (37.5)
A. flavus	34 (23.4)	10 (28.6)	7 (33.3)	3 (13.0)	2 (12.5)	2 (12.5)	7 (38.9)	3 (18.8)
A. niger	19 (13.1)	5 (14.3)	4 (19.0)	2 (8.7)	1 (6.3)	2 (12.5)	3 (16.7)	2 (12.5)
A. terreus	14 (9.7)	0 (0.0)	0 (0.0)	5 (21.7)	4 (25.0)	4 (25.0)	0 (0.0)	1 (6.3)
A. candidus	6 (4.1)	2 (5.7)	0 (0.0)	0 (0.0)	0 (0.0)	4 (25.0)	0 (0.0)	0 (0.0)
A. penicillioids	5 (3.4)	2 (5.7)	1 (4.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	0 (0.0)
A. ustus	1 (0.7)	1 (2.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rhizopus spp.	33 (22.8)	6 (17.1)	7 (33.3)	3 (13.0)	4 (25.0)	4 (25.0)	3 (16.7)	6 (37.5)
Penicillium spp.	19 (13.1)	5 (14.3)	2 (9.5)	7 (30.4)	0 (0.0)	0 (0.0)	1 (5.6)	4 (25.0)
Mucor spp.	7 (4.8)	4 (11.4)	0 (0.0)	0 (0.0)	3 (18.8)	0 (0.0)	0 (0.0)	0 (0.0)
Alternaria spp.	5 (3.4)	0 (0.0)	0 (0.0)	3 (13.0)	2 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)
Ulocladium spp.	2 (1.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	0 (0.0)
Total	**145 (100)**	**35 (24.1)**	**21 (14.5)**	**23 (15.9)**	**16 (11.0)**	**16 (11.0)**	**18 (12.4)**	**16 (11.0)**

N: number of examined isolates.

TABLE 4. Incidence of toxigenic *A. flavus* strains in the examined samples

Type of samples	Number of *A. flavus* isolates	Toxigenic *A. flavus* strains	
		No.	%
Ras cheese	10	10	100.0
Plain yogurt	7	6	85.7
Flavored yogurt	3	2	66.7
Milk powder	2	1	50.0
Milk-based cereals	2	0	0.0
Cheese analogue	7	7	100.0
Cream analogue	3	3	100.0
Total	**34**	**29**	**85.3**

TABLE 5. Existence of AFM_1 in the examined dairy products (n=150)

Sample type	Positive samples	Concentration (ng/kg)		Exceed (ES) and (EC) regulations*
	No. (%)	Range	Mean ± SEM	No. (%)
Ras cheese	30 (100.0)	37.3 – 193.6	89.05 ± 6.21	24 (80.0)
Plain yogurt	21 (70.0)	32.1 – 114.7	63.42 ± 5.04	11 (52.4)
Flavored yogurt	17 (56.7)	27.3 – 80.2	48.71 ± 2.65	6 (35.3)
Milk powder	19 (63.3)	23.2 – 75.6	51.70 ± 2.55	8 (42.1)
Milk-based cereals	14 (46.7)	14.2 – 62.1	35.22 ± 3.36	6 (42.9)

n: number of the examined samples SEM: standard error of the mean
*ES: The Egyptian Standards (ES 7136/2010): AFM1 <50 ng/kg.
*EC: European Commission (EC 165/2010): AFM1 <25 ng/kg (infant formulae), <50 ng/kg (dairy products).

TABLE 6. Concentrations of aflatoxin M_1 in the examined dairy products

Sample type	Range of aflatoxin M_1 (ng/kg)				
	< 50 No. (%)	50–80 No. (%)	81–120 No. (%)	121–150 No. (%)	151–200 No. (%)
Ras cheese	6 (20.0)	3 (10.0)	17 (56.7)	3 (10.0)	1 (3.3)
Plain yogurt	19 (63.3)	5 (16.7)	6 (20.0)	0 (0.0)	0 (0.0)
Flavored yogurt	24 (80.0)	5 (16.7)	1 (3.3)	0 (0.0)	0 (0.0)
Milk powder	22 (73.3)	8 (26.7)	0 (0.0)	0 (0.0)	0 (0.0)
Milk-based cereals	24 (80.0)	6 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)

No.: number of samples.

TABLE 7. Total aflatoxins concentrations in dairy substitutes contaminated with molds and their conformity with EC and US regulations

Sample type	Total mold-positive samples	Aflatoxin Positive samples	Concentration (ppb)		Exceed (EC) regulations *	Exceed (US) regulations **
		No. (%)	Range	Mean ± SEM	No. (%)	No. (%)
Cheese analogue	15	7 (46.6)	7.41 - 25.13	12.32 ± 1.58	4 (57.1)	4 (57.1)
Cream analogue	10	3 (30.0)	6.23 - 11.21	7.46 ± 0.45	(0.0)	(0.0)

SEM: standard error of mean
*EC (EC 165/2010): European Commission regulation: 4-15 ppb for food.
**US (FDA 2021): United States regulations' maximum level: 20 ppb for food.

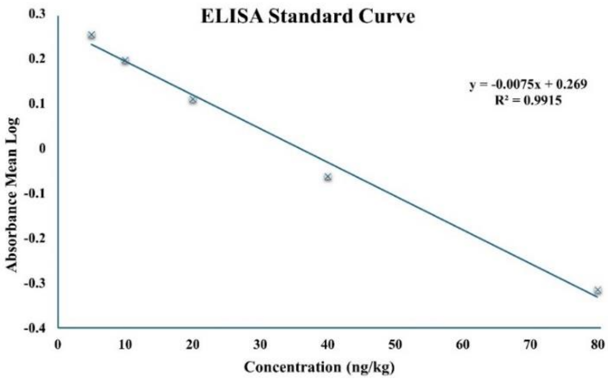

Fig. 1. Calibration curve for AFM_1.

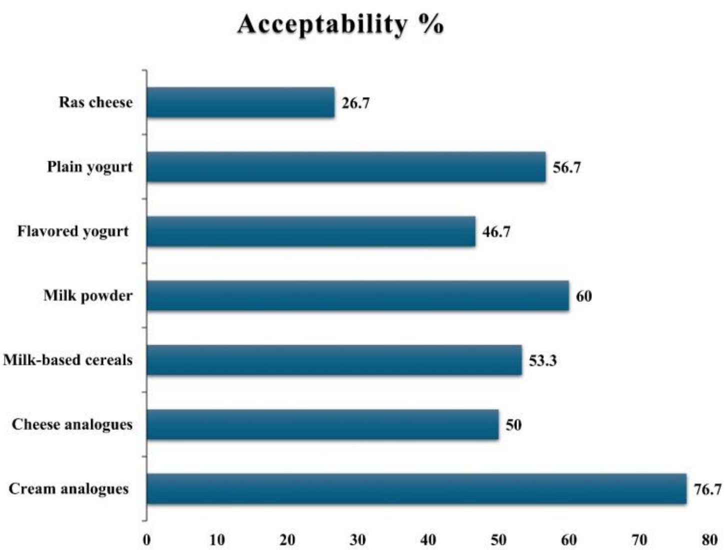

Fig. 2. Acceptability % of the examined products according to the ES.

Ras cheese [63]: Mold < 10 cells/g.
Plain yogurt and flavored yogurt [64]: Mold < 100 cells/g.
Milk powder [65]: Mold < 10 cells/g.
Milk-based cereals [66]: Mold < 10 cells/g.
Cheese analogues [67]: Mold < 10 cells/g.
Cream analogues [68]: Mold < 100 cells/g.

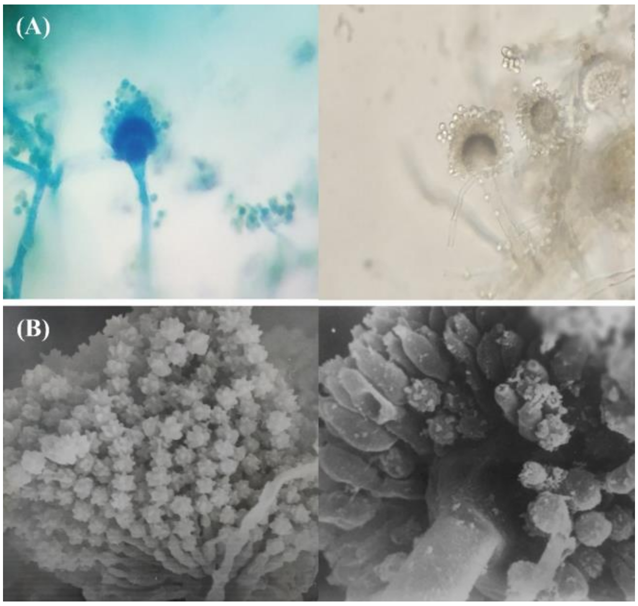

Fig. 3. (A) Light microscopy images (40x) of *A. flavus* conidiophores and conidia stained with lactophenol cotton blue (left) and observed under wet mount preparation (right). (B) Scanning electron micrographs of *A. flavus* conidiophores and conidial heads at different magnifications.

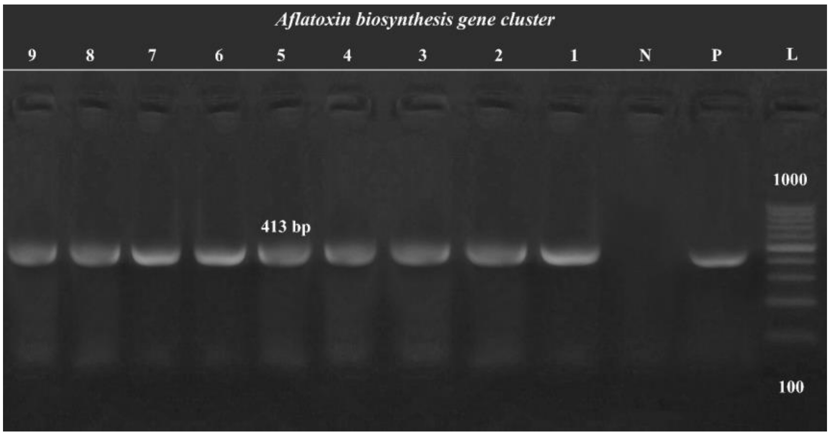

Fig. 4. Agarose gel electrophoresis of PCR products of *A. flavus*. Lane L: 100bp DNA ladder. Lane P: Control Positive. Lane N: Control Negative. Lane 1-9: positive isolates of *Aspergillus flavus* (413bp).

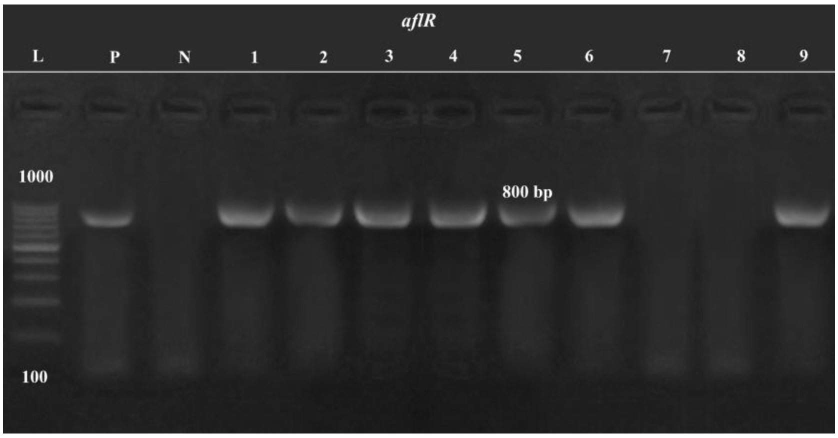

Fig. 5. PCR-amplified products of Aflatoxin B_1 *aflR* gene. Lane L: 100bp DNA ladder. Lane P: Control Positive. Lane N: Control Negative. Lane 1-6 and 9: *A. flavus* isolates harboring Aflatoxin B_1 *aflR* gene (800bp). Lane 7 and 8: Absence of amplification of *aflR* gene.

References

1. Awasti, N. and Anand, S., The Role of Yeast and Molds in Dairy Industry: An Update, in *Dairy Processing: Advanced Research to Applications*, Minj, J., Sudhakaran, V., A, and Kumari, A., Springer: Singapore. pp. 243-262 (2020).

2. Adam, A.H., Aly, S.A. and Saad, M.F. Microbiological quality of some commercial dairy products and cheese analogues focused on Mideast. *Journal of the Hellenic Veterinary Medical Society*, **75**(1), 6945-6958 (2024). DOI: 10.12681/jhvms.33549.

3. Furmanek, Ł., Czarnota, P. and Seaward, M.R.D. The effect of lichen secondary metabolites on Aspergillus fungi. *Archives of Microbiology*, **204**(1), 100 (2021). DOI: 10.1007/s00203-021-02649-0.

4. Martin, N.H., Snyder, A. and Wiedmann, M., Yeasts and Molds: Spoilage Mold in Dairy Products, in *Encyclopedia of Dairy Sciences*, Paul L.H. McSweeney and McNamara, J.P., Academic Press: Oxford. pp. 607-610 (2022).

5. Nafisatu, B., Mabel, K. and Oluwaseyi, A. Aflatoxin M1 (Aspergillus parasiticus, flavus) Occurrences in Milk and Milk Products and Its Possible Health Effects. *Advances in Microbiology*, **10**(10), 509-524 (2020). DOI: 10.4236/aim.2020.1010038.

6. Garnier, L., Valence, F. and Mounier, J. Diversity and Control of Spoilage Fungi in Dairy Products: An Update. *Microorganisms*, **5**(3), 42 (2017). DOI: 10.3390/microorganisms5030042.

7. Jiang, Y., Ogunade, I.M., Vyas, D. and Adesogan, A.T. Aflatoxin in Dairy Cows: Toxicity, Occurrence in Feedstuffs and Milk and Dietary Mitigation Strategies. *Toxins (Basel)*, **13**(4), 283 (2021). DOI: 10.3390/toxins13040283.

8. Jakšić, S., Živkov Baloš, M., Prodanov Radulović, J., Jajić, I., Krstović, S., Stojanov, I. and Mašić, Z. Aflatoxin m1 in milk and assessing the possibility of its occurrence in milk products. *Archives of Veterinary Medicine*, **10**(1), 3-49 (2019). DOI: 10.46784/e-avm.v10i1.80.

9. Malissiova, E., Tsinopoulou, G., Gerovasileiou, E.S., Meleti, E., Soultani, G., Koureas, M., Maisoglou, I. and Manouras, A. A 20-Year Data Review on the Occurrence of Aflatoxin M1 in Milk and Dairy Products in Mediterranean Countries—Current Situation and Exposure Risks. *Dairy*, **5**(3), 491-514 (2024). DOI: 10.3390/dairy5030038

10. IARC. International Agency for Research on Cancer. Monographs on the Evaluation of Carcinogenic Risks to Humans; Chemical agents and related occupations. No. 100F. *International Agency for Research on Cancer*, Lyon (FR), (2012).

11. Iqbal, S.Z., Waqas, M. and Latif, S. Incidence of Aflatoxin M1 in Milk and Milk Products from Punjab, Pakistan, and Estimation of Dietary Intake. *Dairy*. **3**(3), 577-586 (2022). DOI: 10.3390/dairy3030041.

12. Udomkun, P., Mutegi, C., Wossen, T., Atehnkeng, J., Nabahungu, N.L., Njukwe, E., Vanlauwe, B. and Bandyopadhyay, R. Occurrence of aflatoxin in agricultural produce from local markets in Burundi and Eastern Democratic Republic of Congo. *Food Science and Nutrition*, **6**(8), 2227-2238 (2018). DOI: 10.1002/fsn3.787.

13. EC. European Commission Regulation (EU). No. 165 of 26 February 2010,amending Regulation (EC) No 1881/2006 Setting Maximum Levels for Certain Contaminants in Foodstuffs as Regards Aflatoxins. Official Journal of the European Union. 8-12 (165/2010).

14. FDA. (US Food and Drug Administration). 2021. Compliance Policy Guide Sec. 555.400. In Aflatoxins in Human Food: Guidance for FDA. (2021).

15. ES. (Egyptian standards), 2010. Maximum levels for certain contaminants in foodstuff. (2018-Commission Regulation (EC) No 1881/2006). Egyptian Organization for Standardization. (7136/2010).

16. Kumar, P., Mahato, D.K., Kamle, M., Mohanta, T.K. and Kang, S.G. Aflatoxins: A Global Concern for Food Safety, Human Health and Their Management. *Front. Microbiol*., **7**, 2170 (2017). DOI: 10.3389/fmicb.2016.02170.

17. Taylor, T.M., Sofos, J.N., Bodnaruk, P. and Acuff , G.R., Sampling plans, sample collection, shipment, and preparation for analysis, in *Compendium of methods for the microbiological examination of foods*American Public Health Association: Washington, DC., USA. pp. 13-25 (2015).

18. ISO. Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of yeasts and moulds - Part 1: Colony count technique in products with water activity greater than 0.95, by means of the colony count technique at 25°C plus or minus 1 °C. 8 (21527-1:2008).

19. Sciortino Jr, C.V. *Atlas of clinically important fungi*. John Wiley & Sons, Inc, (2017). DOI: 10.1002/9781119069720.

20. Samson, R.A., Visagie, C.M., Houbraken, J., Hong, S.B., Hubka, V., Klaassen, C.H., Perrone, G., Seifert, K.A., Susca, A., Tanney, J.B., Varga, J., Kocsubé, S., Szigeti, G., Yaguchi, T. and Frisvad, J.C. Phylogeny, identification and nomenclature of the genus Aspergillus. *Studies in Mycology*, **78**, 141-173 (2014). DOI: 10.1016/j.simyco.2014.07.004.

21. Aly, S.A., Seham, M.H., Ragaa, S.H., Mahrus, A.R. and Diekmann, H., *Studies on mycotoxins in milk and some dairy products*, Cairo university (1999).

22. El-Kadi, S., Elfadaly, H. and El-Gayar, E.-S. Scanning Electron Microscopy of Fungi Isolated from Some Cake Samples. *International Journal of Microbiology and Application*, **5**(3), 50-55 (2018).

23. Bintvihok, A., Treebonmuang, S., Srisakwattana, K., Nuanchun, W., Patthanachai, K. and Usawang, S. A Rapid and Sensitive Detection of Aflatoxin-producing Fungus Using an Optimized Polymerase Chain

Reaction (PCR). *Toxicological Research*, **32**(1), 81-87 (2016). DOI: 10.5487/tr.2016.32.1.081.

24. Trinh, H.L.T., Anh, D.T., Thong, P.M. and Hue, N.T. A Simple PCR for Detection of Aspergillus flavus in Infected Food. *Quality Assurance and Safety of Crops & Foods*. **7**(3), 1-9 (2015). DOI: 10.3920/QAS2013.0308.

25. ISO. Milk and Milk Product Guidelines for a Standardized Description of Competitive Enzyme Immunoassay. Determination of Aflatoxin M1. International Organization for Standardization. (14675:2003).

26. Ahmed, E.A., Al-Kahtani, M.M., Eldiasty, E.M., Ahmed, S.A., Saber, H., Abbas, A.M., Diab, H.M., Alshehri, M.A., Elmansi, A.A. and Hussein, M.A. Diversity of Toxigenic Molds and Mycotoxins Isolated from Dairy Products: Antifungal Activity of Egyptian Marine Algae on Aspergillus and Candida Species. *Journal of Pure and Applied Microbiology*, **14**(1), 215-232 (2020). DOI: 10.22207/JPAM.14.1.23.

27. Worku, K.F., Tefera, A.T. and Tuji, F.A. Comparative Analysis of Microbial Load of Commercially Prepared and Traditionally Homemade Yoghurt (Ergo) Retailed in Addis Ababa. *Advances in Life Sciences*, **5**(3), 58-63 (2015). DOI: 10.5923/j.als.20150503.02.

28. Keta, J.N., Suberu, H.A., Aliero, A.A., Mohammed, N.K., Anas, H. and Mubarak, A. Evaluation of Fungi Species from Commercial Yoghurts in Birnin Kebbi, Kebbi State Nigeria. *Equity Journal of Science and Technology*, **6**(1), 72-77 (2019).

29. Aman, I.M., Al-Hawary, I.I., Elewa, S.M., El-Kassas, W.M. and El-Magd, M.A. Microbiological evaluation of some Egyptian fermented dairy products. *Journal of the Hellenic Veterinary Medical Society*, **72**(2), 2889-2896 (2021). DOI: 10.12681/jhvms.27528.

30. Pal, M. Spoilage of Dairy Products due to Fungi. *Beverage and Food World*, **41**(7), 37-40 (2014).

31. Abdelkhalek, A., Elsherbini, M., Eletriby, D. and Sadak, A. Quality assessment of imported powder milk at Mansoura city, Egypt. *Journal of Advanced Veterinary and Animal Research*. **3**(1), 75-78 (2016). DOI: 10.5455/javar.2016.c122.

32. Abdel-Hameed, Z., El-Gendi, M. and Oraby, N. Detection of aflatoxins in UHT and powdered flavored milk. *Assiut Veterinary Medical Journal*, **65**(162), 1-6 (2019). DOI: 10.21608/avmj.2019.166579.

33. Ibrahim, A.S., Saad, M.F. and Hafiz, N.M. Safety and quality aspects of whole and skimmed milk powders. *Acta Scientiarum Polonorum Technologia Alimentaria*., **20**(2), 165-177 (2021). DOI: 10.17306/j.Afs.0874.

34. Aidoo, K., Mohamed, S., Candlish, A., Tester, R. and Elgerbi, A. Occurrence of Fungi and Mycotoxins in Some Commercial Baby Foods in North Africa. *Food and Nutrition Sciences*, **2**, 751-758 (2011). DOI: 10.4236/fns.2011.27103.

35. Ajayi, O.A. and Okiti, U.O. Nutritional and microbial quality of selected commercially available powdered infant formula during the Covid-19 pandemic. *IOP Conference Series: Earth and Environmental Science*, **1219**(1), 012021 (2023). DOI: 10.1088/1755-1315/1219/1/012021.

36. Adam, A.H., Aly, S.A. and Saad, M.F. Evaluation of microbial quality and safety of selected dairy products with special focus on toxigenic genes of Bacillus cereus. *Mljekarstvo*. **71**(4), 257-268 (2021a). DOI: 10.15567/mljekarstvo.2021.0405.

37. Shi, C. and Maktabdar, M. Lactic Acid Bacteria as Biopreservation Against Spoilage Molds in Dairy Products - A Review. *Front Microbiol*., **12**, 819684 (2021). DOI: 10.3389/fmicb.2021.819684.

38. Hamad, M.N., Eman, M.T. and Mohamed, W.M. Effect of fortification palm oil with some spices on physico-chemical composition, microbiological analysis, sensory evaluation and economic study of Tallaga-like cheese. *Indian Journal of Dairy Science*, **70**(1), 23-31 (2017).

39. Mohamed, S., Abdou, M., Elbarbary, A. and Elbaba, H. Assessment of microbiological quality in some cheese varieties in Egypt. *Benha Veterinary Medical Journal*, **36**(1), 164-174 (2019). DOI: 10.21608/bvmj.2019.103408.

40. El-gendi, M.M.N. and El-shreef, L.M.T.A. Microbiological investigations of some dried mixes of dairy desserts sold in assiut city. *Assiut Veterinary Medical Journal*, **59**(137), 180-188 (2013). DOI: 10.21608/avmj.2013.171582.

41. Ibrahim, G.A., Sharaf, O.M., and El-ssayad, M.F. Prevalence of Fungal Spoilage in Local Tallaga Cheese and Applicate Novel Antimicrobial "PHR" to Extend Its Shelf-Life. *Egyptian Journal of Chemistry*, **65**(131), 1551-1557 (2022). DOI: 10.21608/ejchem.2022.140721.6158.

42. Seddek, N., Gomah, N. and Osman, D. Fungal Flora Contaminating Egyptian Ras Cheese with Reference to Their Toxins and Enzymes. *Food Science and Technology*, **4**(4), 64-68 (2016). DOI: 10.13189/fst.2016.040403.

43. Fetouh, M., Ibrahim, E., ElBarbary, H. and Maarouf, A. Isolation and Genotypic Identification of Some Spoilage and Pathogenic Microbes from Yogurt. *Benha Veterinary Medical Journal*, **43**(1), 123-128 (2022). DOI: 10.21608/bvmj.2022.147816.1539.

44. Oyeyipo, O., Oyeyipo, F. and Ayah, I. Bio-Burden and Antibacterial Susceptibility Pattern of Repacked Milk Powder on Sale in Ogun State Markets, South-Western Nigeria. *Sokoto Journal of Medical Laboratory Science*, **2**(3), 156-166 (2017).

45. Salim, D.A., Rady, F.M. and EL-toukhy, E.I. Fungal Contamination of Some Local Dairy Products and extent Production of Aflatoxins. *Life Science Journal*, **17**(7), 7-13 (2020). DOI: 10.7537/marslsj170720.02.

46. Rahman, H.U., Yue, X., Yu, Q., Zhang, W., Zhang, Q. and Li, P. Current PCR-based methods for the detection of mycotoxigenic fungi in complex food and feed matrices. *World Mycotoxin Journal*, **13**(2), 139-150 (2020). DOI: 10.3920/WMJ2019.2455.

47. Amer, A.A. and Ibrahim, M.A.E. Determination of aflatoxin M1 in raw milk and traditional cheeses retailed in Egyptian markets. *Journal of Toxicology and Environmental Health Sciences*, **2**(4), 50-53 (2010).

48. Camaj Ibrahimi, A., Berisha, B., Haziri, A., Camaj Isa, A., Sopjani, H., Muriqi, S., Shala, N. and Hoxha, I. Occurrence of Aflatoxin M1 in yogurt samples found in markets in Kosovo during spring 2023. *Veterinarska Stanica*, **55**(3), 301-310 (2023). DOI: 10.46419/vs.55.3.6.

49. Corassin, C., Borowsky, A., Sher, A., Rosim, R. and Oliveira, C. Occurrence of Aflatoxin M1 in Milk and Dairy Products Traded in São Paulo, Brazil: An Update. *Dairy*, **3**(4), 842-848 (2022). DOI: 10.3390/dairy3040057.

50. Iqbal, S.Z., Asi, M.R. and Malik, N. The seasonal variation of aflatoxin M1 in milk and dairy products and assessment of dietary intake in Punjab, Pakistan. *Food Control*, **79**, 292-296 (2017). DOI: 10.1016/j.foodcont.2017.04.015.

51. Heshmati, A., Mozaffari Nejad, A.S.M. and Ghyasvand, T. The Occurrence and Risk Assessment of Aflatoxin M1 in Yoghurt Samples from Hamadan, Iran. *The Open Public Health Journal*, **13**, 512-517 (2020). DOI: 10.2174/1874944502013010512.

52. Alahlah, N., El Maadoudi, M., Bouchriti, N., Triqui, R. and Bougtaib, H. Aflatoxin M1 in UHT and powder milk marketed in the northern area of Morocco. *Food Control*, **114**, 107262 (2020). DOI: 10.1016/j.foodcont.2020.107262.

53. Marimón Sibaja, K.V., Gonçalves, K.D.M., Garcia, S.O., Feltrin, A.C.P., Nogueira, W.V., Badiale-Furlong, E. and Garda-Buffon, J. Aflatoxin M1 and B1 in Colombian milk powder and estimated risk exposure. *Food Addit. Contam. Part B Surveill*. **12**(2), 97-104 (2019). DOI: 10.1080/19393210.2019.1567611.

54. Murshed, S. Evaluation and Assessment of Aflatoxin M1 in Milk and Milk Products in Yemen Using High-Performance Liquid Chromatography. *Journal of Food Quality*, **2020**(1), 8839060 (2020). DOI: 10.1155/2020/8839060.

55. Elaridi, J., Dimassi, H. and Hassan, H. Aflatoxin M1 and ochratoxin A in baby formulae marketed in Lebanon: Occurrence and safety evaluation. *Food Control*, **106**, 106680 (2019). DOI: 10.1016/j.foodcont.2019.06.006.

56. Malissiova, E., Soultani, G., Tsokana, K., Alexandraki, M. and Manouras, A. Exposure assessment on aflatoxin M1 from milk and dairy products-relation to public health. *Clinical Nutrition ESPEN*. **47**, 189-193 (2022). DOI: 10.1016/j.clnesp.2021.12.017.

57. Adam, A.H., Aly, S.A., Sayed, R.H.S. and Saad, M.F. Occurrence of Aflatoxin M1 in Cheese and Yoghurt Marketed at El-Fayoum Province, Egypt. *International Journal of Dairy Science*., **16**(4), 146-152 (2021b). DOI: 10.3923/ijds.2021.146.152.

58. Sabatelli, S., Gambi, L., Baiguera, C., Paterlini, F., Mami, F.L., Uboldi, L., Daminelli, P. and Biancardi, A. Assessment of aflatoxin M1 enrichment factor in cheese produced with naturally contaminated milk. *Italian Journal of Food Safety*., **12**(2), 11123 (2023). DOI: 10.4081/ijfs.2023.11123.

59. Campagnollo, F.B., Ganev, K.C., Khaneghah, A.M., Portela, J.B., Cruz, A.G., Granato, D., Corassin, C.H., Oliveira, C.A.F. and Sant'Ana, A.S. The occurrence and effect of unit operations for dairy products processing on the fate of aflatoxin M1: A review. *Food Control*., **68**, 310-329 (2016). DOI: 10.1016/j.foodcont.2016.04.007.

60. Braun, D., Eiser, M., Puntscher, H., Marko, D. and Warth, B. Natural contaminants in infant food: The case of regulated and emerging mycotoxins. *Food Control*, **123**, 107676 (2021). DOI: 10.1016/j.foodcont.2020.107676.

61. Embaby, E.M., Awni, N.M., Abdel-Galil, M.M. and El-Gendy, H.I. Distribution of fungi and mycotoxins associated some Foods. *Middle East Journal of Applied Sciences*, **5**(3), 734-741 (2015).

62. Abrehame, S., Manoj, V.R., Hailu, M., Chen, Y.-Y., Lin, Y.-C. and Chen, Y.-P. Aflatoxins: Source, Detection, Clinical Features and Prevention. *Processes*, **11**(1), 204 (2023). DOI: 10.3390/pr11010204.

63. ES. (Egyptian standards), 2005. Hard cheese part: 5 Ras cheese. Egyptian Organization for Standardization. (1007-5/2005).

64. ES. (Egyptian standards), 2024. Fermented milks. Egyptian Organization for Standardization. (8042/2024).

65. ES. (Egyptian standards), 2023. Milk powder and Cream powder. Egyptian Organization for Standardization. (8073/2023).

66. ES. (Egyptian standards), 2017. Processed cereal-based foods for infants. Egyptian Organization for Standardization. (3284/2017).

67. ES. (Egyptian standards), 2005. Soft cheese with vegetable fats. Egyptian Organization for Standardization. (1867/2005).

68. ES. (Egyptian standards), 2005. Whipping cream powder with vegetable fats. Egyptian Organization for Standardization. (1599/2005).

تنوع العفن السام والأفلاتوكسينات في أنواع مختلفة من منتجات الألبان وبدائلها

عبدالكريم حامد آدم[1]* ، سلوى أحمد علي[1] ، مينا فؤاد سعد[1] ، رشا محمود حمزة سيد الأهل[2] و أمل السيد إبراهيم[1]

[1] قسم الرقابة الصحية علي الأغذية، كلية الطب البيطري، جامعة القاهرة، مصر.

[2] قسم الفطريات والسموم الفطرية، مركز بحوث صحة الحيوان، مركز البحوث الزراعية فرع الجيزة، مصر.

الملخص

على الرغم من أن منتجات الألبان أكثر عرضة لنمو العفن، فإن بدائل الألبان معرضة أيضًا للخطر. تمتد مشكلة تلوث العفن في منتجات الألبان إلى ما هو أبعد من مخاوف الجودة، لتشمل مخاوف أوسع نطاقًا تتعلق بسلامة الأغذية والصحة العامة. وفي هذا الصدد، تم إجراء تحقيق في نسبة حدوث العفن وتحديد العفن السائد ومراقبة مستويات الأفلاتوكسين عبر اختبار الاليزا في عدد 210 عينة من منتجات الألبان وبدائل الألبان التي تم الحصول عليها من أسواق مختلفة في محافظة الفيوم، مصر. أظهرت النتائج أعلى مستوى تلوث بالعفن في الجبن الرومي بنسبة (73.3٪)، مع أدنى مستوى للتوافق مع المعايير المصرية، علاوة على ذلك، تم تسجيل أدنى مستوى للتلوث (33.3٪) وأعلى مستوى للقبول في عينات نظير الكريمة. كان هناك فرق معنوية (p<0.05) بين متوسط قيم تلوث الجبن الرومي وعينات نظير الجبن، إلى جانب متوسطات عينات الزبادي العادي والزبادي المنكه. كما أظهرت النتائج ان عائلة الاسبرجلس كانت الأكثر عزلًا بنسبة (54.5٪)، يليه أنواع أخرى مثل الريزوبس (22.8%)، بنسيليوم (13.1%)، ميوكور (4.8%)، الترناريا (3.4%)، و اولوكلاديوم بنسبة (1.4%). أظهر توصيف معزولات الاسبرجلس فلافس بواسطة اختبار تفاعل البلمرة المتسلسل أن 100.0٪ من العزلات تم تأكيدها، بينما احتوت نسبة 85.3٪ من المعزولات على جين الأفلاتوكسين B_1 *aflR*. تم الكشف عن الأفلاتوكسين M_1 في جميع عينات الجبن الرومي التي تم تحليلها، 70.0% زبادي عادي، 56.7% زبادي منكه، 63.3% حليب مجفف، و46.7% حبوب أساسها الحليب، بمتوسط قيم 89.05±6.21، 63.42±5.04، 48.71±2.65، 51.70±2.55، و35.22±3.36 نانوجرام/كجم، على التوالي. تم قياس إجمالي الأفلاتوكسين في عينات نظيرة للجبن ونظيرة للكريمة بمتوسط مستويات 12.32±1.58 و7.46±0.45 جزء في المليار على التوالي. احتوى جزء كبير من العينات التي تم تقييمها ليس فقط على مستويات متفاوتة من عفن الاسبرجلس ولكن أيضًا على الأفلاتوكسينات الخاصة بها. وهذا يستلزم المراقبة المتكررة لإجمالي الأفلاتوكسين في بدائل الألبان و الأفلاتوكسين M_1 في منتجات الألبان. علاوة على ذلك، فإن تطبيق تدابير الرقابة الصارمة خلال المراحل المختلفة لإنتاج ومعالجة هذه المنتجات، فضلاً عن التعامل السليم مع الأعلاف الحيوانية وتخزينها، من شأنه أن يقلل من نمو العفن.

الكلمات الدالة: الاسبرجلس فلافس، الأفلاتوكسين M_1، مجموع الأفلاتوكسينات، الحبوب القائمة على الحليب، بدائل الألبان، اختبار الاليزا.