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Fungal Contamination of Some Local Dairy Products and extent Production of Aflatoxins

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Abstract: This study conducted in Egypt aims to investigate the cheese and yogurt products' safety in relation to the HACCP (Hazard Analysis and Critical Control Point) – a matter of high concern in food supplies. For collection of data, 30 samples from each Feta, Damietta, Kareish cheese and yogurt were randomly gathered from different dairy shops and supermarkets located in Menofia governorate. All these 120 samples were subjected to mycological and molecular identification. The mycological examination revealed that the contaminated samples of Feta cheese, Domietti cheese, Kareish cheese and Yoghurt with molds were 76.67,100, 86.67 and 80%, respectively with mean values of 2.47±0.22, 3.27±0.33, 2.89±0.34 and 2.65±0.35 (log cfu/g), correspondingly. On the other hand, the contaminated samples of Feta cheese, Domietti cheese, Kareish cheese and Yoghurt with yeasts came to be 76.67, 100,100 and 83.33% with mean values of 2.94 ±0.26, 3.43 ±0.50, 4.46±0.59 and 3.47±0.73(log cfu/g), respectively. The identified molds from the examined samples of dairy products of a varying percentage were penicillum, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Aspergillus terreus, Cladosporium, Alternaria, Rhizopus, and Mucor. Detecting Aspergillus flavus was based on polymerase chain reaction by using specific primers depend on the aflatoxin biosynthesis gene clusters. All A. flavus isolates obtained were positive for the target gene. The findings demonstrated that high percentages of the samples examined did not comply with Egyptian standards, which constituted a high risk to consumer health. For producers as well as the HACCP system (Hazard Analysis and Critical Control Point) thus, safety measures and educational programs must be implemented.
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1. Introduction

Milk and milk products are essential components of human diet in many parts of the world due to its high nutritional value [1]. However, these products foster a suitable environment for growth and multiplication of microorganisms such as fungi because of their moisture content and richness in several nutrients [2]. Fungi is primarily responsible for visible and invisible changes, like unpleasant odors and undesirable flavor, and lead to food waste and economic losses [3]. Besides, unsanitary conditions, contaminated environment and human factors also cause contamination of milk and its products at any step either in the farms or in the manufacturing units [4-6]. Contamination of milk products with *Aspergillus*, *Fusarium*, and *Penicillium* species has a public health hazard owing to their ability to produce mycotoxins that are harmful to human health [7,8].

Aflatoxins are the most significant mycotoxins in foods around the world and can cause acute and chronic toxicity in animals and humans. Furthermore, the high carcinogenicity of these mycotoxins supports all efforts in food monitoring and control [9]. *A. flavus* and *A. parasiticus* are the major aflatoxins - producing fungi that belong to *Aspergillus*, and other species such as *A. nomius*, *A. pseudotamarii*, *A. bombycis*, *A. ochraceus* [10, 11]. A. flavus is also the main producer of Aflatoxin B1, the best known potent carcinogen in the liver and Aflatoxin B2 [9]. AFM1 in milk resulting from the conversion of aflatoxin B1 (AFB1) by dairy animals feeds on the contaminated feedstuffs, to AFM1, which then passes to their urine and milk [12].

Over 60 species of yeast have been reported as spoilage agents of dairy products and heat-treated products [12]. The Aflatoxins biosynthesis pathway includes about 25 genes clustered in a region of 70 kb of DNA [13]. Recently, PCR detection of the presence or expression of Aflatoxin biosynthetic gene has been used for the diagnosis of aflatoxigenic fungi which molecularly identified for the presence of this gene [14].

The study aimed to monitor the distribution of fungi in various types of cheese and yoghurt commonly consumed in Egypt and to identify isolated fungal strains. DNA-based detection methods such as PCR are more effective than conventional methods for the detection and identification of fungi in foods that are time-consuming and laborious in this direction.

2. Material and Methods

Collection of sample:

A total of one hundred and twenty random samples from Feta, Domietti and Kareish cheese and Yoghurt (30 samples of each) were collected from different dairy shops and supermarkets in Menofia governorate. All the samples were transported to the laboratory in sterile airtight jars with minimal delay in preparation and mycological examination was carried out.

Enumeration, Isolation, and Identification of fungi

Ten grams of each sample was homogenized in a stomacher bag with a 90 ml sterile solution of 0.2 % sodium citrate. One ml of two successive dilutions was put into petri dishes, then poured Potato dextrose agar (PDA) supplemented with antibiotics and mixed well then left to solidify. The Petri dishes had been incubated for 5 days at 25±2 ° C. Then we counted and picked up single colonies of fungi.

The mould counts were determined [15], and then the colonies were transferred to PDA plates to obtain pure fungal isolates [16]. The fungal isolates were conserved at 5 ° C for use on PDA slants. The fungal isolates were sub-cultured on new PDA slants before use, and incubated for 5 days at 25 ° C. Mounts of pure fungal isolates have been identified in PDA medium by morphological characteristics of colonies. Furthermore, the vegetative and reproduction strictness and morphological features according to standard identification keys and the most documented atlas keys in fungal identification [17].

The collected data were presented as a mean and standard deviation and a one-way variance analysis (ANOVA) $P<0.05$ was performed to assess the correlation between the means of different mold and yeast counts in the different products. SPSS (Version16.0) conducted all statistical calculations.

Polymerase Chain Reaction (PCR)

Extraction of fungal DNA

The extraction of DNA was made using QIAamp DNeasy Plant Mini Kit (Qiagen, Germany, GmbH). Briefly, 100 mg of the *A. Flavus* sample was added to 400 µl Buffer AP1 and 4 µl RNase A stock solution (100 mg/ml), tungsten carbide bead was added to the previous mixture in a 2 ml safe-lock tube. Tubes were placed in the adapter sets, which are attached to the tissue lyser clamps. Disruption occurred in two 1–2 minute high-speed (20–30 Hz) shaking steps. The mixture was incubated at 65 ° C for 10 minutes, and mixed by inverting the tube 2 or 3 times during incubation. Then, 130 µl Buffer P3 was added to the lysate, mixed, and incubated on ice for 5 min. At 14,000 rpm, the lysate was centrifuged for 5 min, then pipetted into the QIA shredder Mini spin column (lilac) put in a 2 ml collection tube, and centrifuged for 2 min at 14,000 rpm. The flow-through fraction from was transferred to a new tube without disturbing the cell-debris pellet and then applied to the silica column. The lysate was then washed and centrifuged as recommended by the manufacturer. With 50 µl of elution buffer provided in the kit, nucleic acid was eluted.

DNA Amplification:

Primers were used in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed as shown in the following table in an applied biosystem 2720 thermal cycler.

Oligonucleotide primers, target genes, amplicon sizes and cycling conditions for conventional PCR (Primers used were supplied from Bio basic Canada).

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Aflatoxin biosynthesis gene cluster	GGTGGTGAAGAAGTCTATCTAAGG AAGGCATAAAGGGTGTGGAG	413	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	[18]
Aflatoxin B1 aflR	AAC CGC ATC CAC AAT CTC AT AGT GCA GTT CGC TCA GAA CA	800	94˚C 5 min.	94˚C 30 sec.	50˚C 1.25 min.	72˚C 1.40 min.	72˚C 10 min.	[19]

Analysis of the PCR Products.

PCR products were separated by electrophoresis using 5V / cm gradients on 1.5 % agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature. 15 µl of the products were loaded into each gel slot for gel analysis. The gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) and generuler 100 bp DNA ladder (Fermentas, Thermo) were used to determine the size of the fragments. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

3. Results

Table 1: Statistical analytical results of molds count for samples of the examined dairy products (Total mold count / g) (n=30).

Types of dairy products samples	Positive samples		Log cfu /g		
	No	%	Min	Max	Mean ±SD
Feta cheese	23	76.67	2.00	2.76	2.47 ± 0.22^{abC}
Domietti cheese	30	100	2.85	3.86	3.27 ± 0.33^{Abc}
Kareish cheese	26	86.67	2.30	3.53	2.89 ± 0.34^{aBc}
Yoghurt	24	80	2.00	3.11	2.65 ± 0.35^{a}

*Min, Max, and Mean±SD expressed by $\log_{10}$ cfu/ gm. there is a significant difference between the same capital and small letter

Table 2: Statistical analytical results of total yeast count/ g for samples of the examined dairy products (n=30).

Types of dairy products samples	Positive samples		Log cfu /g		Mean ±SD
	No	%	Min	Max	
Feta cheese	23	76.67	2.40	3.34	2.94 ± 0.26^{abC}
Domietti cheese	30	100	2.48	4.11	3.43 ± 0.50^{Abc}
Kareish cheese	30	100	3.72	5.74	4.46 ± 0.59^{aBc}
Yoghurt	25	83.33	2.08	4.40	3.47 ± 0.73^{bc}

*Min, Max, and Mean±SD expressed by $\log_{10}$ cfu/ gm. There is a significant difference between the same capital and small letter

Table 3: Frequency distribution of identified molds in examined samples of dairy products

Isolates of molds	Feta cheese		Domietti cheese		Kareish cheese		Yoghurt	
	No	%	No	%	No	%	No	%
Penicillium spp.	10	38.5	5	20.8	4	20	7	33.3
Aspergillus fumigatus	8	30.8	6	25	5	25	0	0
Aspergillus niger	5	19.2	5	20.8	6	30	5	23.8
Aspergillus flavus	0	0	2	8.3	2	10	1	4.8
Aspergillus terreus	0	0	4	16.7	2	10	0	0
Cladosporium spp	0	0	1	4.2	0	0	2	9.5
Alternaria spp	0	0	1	4.2	0	0	0	0
Rhizopus spp	1	3.8	0	0	0	0	4	19
Mucor spp	2	7.7	0	0	1	5	2	9.5
Total	26	100	24	100	20	100	21	100

Table 4: Summarized results of mycological examination of samples of dairy products compared to the Egyptian standards [20]:

Examined dairy products samples	Molds not more than 10 Cfu/g (23)				Yeasts not more than 4×10^2 Cfu/g (23)			
	Acceptable Samples		Unacceptable Samples		Acceptable Samples		Unacceptable Samples	
	No	%	No	%	No	%	No	%
Feta cheese	7	23.3	23	76.7	11	36.7	19	63.3
Domietti cheese	0	0	30	100	1	3.3	29	96.7
Kareish cheese	4	13.3	26	86.7	0	0	30	100
Yoghurt	6	20	24	80	8	26.7	22	73.3

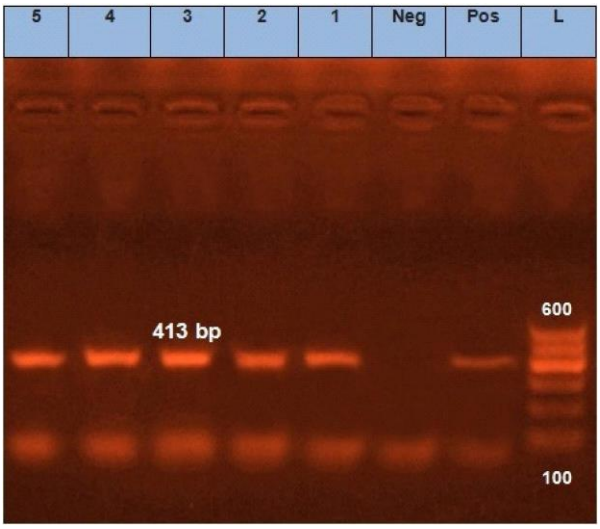

Figure 1: Agarose gel electrophoresis 1.5% showing PCR products of *Aspergillus flavus* Lane (L): is 100bp ladder (QIAGEN, Gmbh) (100-600 bp), lane (Neg): is negative control, lane (Pos): is Positive control and lanes (1-5): are positive isolates of *Aspergillus flavus* (413bp).

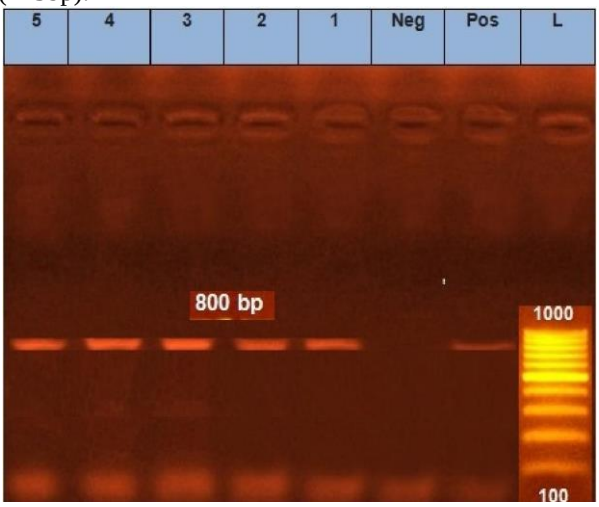

Figure 2: Agarose gel electrophoresis 1.5% showing PCR products of Aflatoxin B1gene (800 bp). Lane (L): is 100bp ladder (QIAGEN, Gmbh) (100-1000 bp), lane (pos): is a positive control, lane (Neg): is a negative control, lanes (1-5): are isolates have Aflatoxin B1gene.

4. Discussion

The presence of yeast and mold in milk and dairy products is not acceptable even if it is found in a few numbers because they result in unwanted changes that perilously undermine the quality of the product [21], 5 to 10% of the world's food production is expected to be lost due to contamination with fungi [9].

Table (1) showed contaminated samples from Feta cheese, Domietti cheese, Kareish cheese, and Yoghurt with molds at percentages of 76.67,100, 86.67 and 80%, respectively with mean values of 2.47±0.22, 3.27±0.33, 2.89±0.34 and 2.65±0.35 (log cfu/g) respectively. A nearly similar result was recorded for Kareish cheese count [22], while some reports in contrast to our study, stated higher count [23] or lower findings [24,25,26]. Other authors were scored lower results for Domietti cheese [22], or higher result for feta cheese [27]. Some reports stated higher results for Yoghurt [28,29]. These results indicate inadequate sanitary measures either during processing [30] or from the milk itself which may be contaminated from the surrounding environment, equipment, handling, and transportation [31].

Table (2) explained the percentage of contaminated samples of Feta cheese, Domietti cheese, Kareish cheese and Yoghurt with yeasts as 76.67, 100,100 and 83.33% respectively with mean values of 2.94 ±0.26, 3.43 ±0.50, 4.46±0.59 and 3.47±0.73(log cfu/g), respectively. This finding agrees with finding for Kareish cheese and Domietti cheese from the same area [32], while it was in parallel to the result of Kareish cheese only from Egypt [27]. Our results were lower than the finding for both Kareish cheese and Domietti cheese from Egypt [22], and also for Yoghurt from Egypt [29] and Pakistan [33]. Yeast that causes spoilage generally originates from contamination of the brine, surface, equipment or ingredients [34, 35] but can also be detected in the air [36]. In one study counts of 1.109 CFU / cm2 in a brining tank were recorded [34].

Identified molds from the examined samples of dairy products were tabulated in table (3). It showed that *penicillum spp* was the highest isolated mold for feta cheese with 38.5% followed by *Aspergillus fumigatus* with 30.8% then *Aspergillus niger* with 19.2% and *Mucor* with 7.7% while *Rhizopus* scored the lowest percentage 3.8. Domietti cheese, *Aspergillus fumigatus* showed the highest percentage of mold isolate (25%) followed by *Penicillum spp* and *Aspergillus niger* with 20.8% then *Aspergillus terreus* with 16.7%. *Aspergillus flavus* represented 8.3% of mold isolates. And the lowest percentage 4.2 of the isolated mold was for *Cladosporium* and *Alternaria spp*. For Kareish cheese the highest isolated mold was *Aspergillus niger* with 30% followed by *Aspergillus fumigatus, penicillum, Aspergillus flavus, Aspergillus terreus* and *Mucor spp* with percentages 25,20,10,10 and 5, respectively. Penicillum spp was the most isolated mold from yoghurt with 33.3% followed by *Aspergillus niger, Rhizopus, Cladosporium, Mucor* and *Aspergillus flavus* with 23.8, 19,9.5,9.5 and 4.8%, correspondingly.

In other studies, some researchers could isolate *pencillium, Aspergillus flavus* and *Rhizopus* from Domity cheese [37], and others were able to isolate *penicillium, Aspergillus fumigatus, Aspergillus terreus*

and *Fonsecaea spp.* from Kareish cheese [23], on the other hand, studies have reported that the isolated moulds were mostly *Cladosporium, Penicillium*, and *Aspergillus* from locally manufactured cheese in Egypt [22].

The frequency percentages of isolated mould species from the examined set – Yoghurt samples from Egypt – were explained as *Cladosporium cladosporioides* with 14 (70 %) and *Penicillium donckii* with 6(30%), but none of the isolated strains belonged to toxigenic species [29]. The frequency distribution of identified molds in examined samples of plain yoghurt revealed that *Penicillium chrysogenum* was the highest isolated (15 %) followed by *Alternaria alternate* (14%) while *Penicillium rubrum* (13%), *Aspergillus niger* (12%) and *Mucor spp.* were the lowest isolated (4%) [38].

Our results, similar to other studies, illustrated that the isolated and identified moulds as; *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* with different percentages from the examined samples of Egyptian cheese. Mainly *Aspergillus* and *Penicillium* were the dominant isolated moulds while *Cladosporium* and *Alternaria* were less dominant [27].

Aspergillus, *penicillium*, *Rhizopus*, *Fusarium*, and *Trichderma* are the most common spoilage species [39]. *Penicillium species can cause softness of the cheese surface [40] and in certain cases have been associated with pulmonary, urinary tract infections and even death.*

Many of the species Aspergillus, Cladosporium, Penicillium and Fusarium have been responsible for kerato-conjunctivitis in humans, while Aspergillus niger causes otomycosis and allergic condition. [41].

In general, toxigenic fungi such as Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius are produced in animal feed [42]. Aflatoxins have sub-acute and chronic effects in humans such as liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis while AFM1 is listed as a probable human carcinogen in Group 2 [43]. Several countries have legislation to regulate AFB1 levels in feed and to recommend maximum permissible AFM1 levels in milk to reduce this risk to consumer health [44]. The human consumption of milk and milk products especially by infants and young children increases the risk of AFM1 exposure [45]. After pasteurization, sterilization, preparation and storage of various dairy products, AFM1 remains stable. [46]. Aflatoxins are the potent known hepatotoxins and carcinogens, and their effects vary with duration of exposure and nutritional status. Acute aflatoxicosis is characterized by acute hepatic necrosis, which eventually results in cirrhosis or carcinoma of the liver. Acute hepatic failure is manifested by hemorrhage, edema, and alteration indigestion, changes to the absorption and/or metabolism of nutrients and mental changes and/or coma [47].

Table (4) explained the percentages of examined dairy products samples which is considered as unacceptable [20] for molds count in Feta cheese, Domietti cheese, Kareish cheese, and Yoghurt were 76.7, 100, 86.7 and 80%, respectively and for yeasts count were 63.3, 96.7,100 and 73.3 %, correspondingly.

Many genetic methods revealed that the genes included in the aflatoxin biosynthetic pathway are accurate, specific, and sensitive detection method. PCR was used for detection of aflatoxigenic strains in grains and foods [48]. In this study, specific primers for aflatoxin regulatory pathway gene (Aflatoxin B1 aflR) were designed, so the detection of aflatoxigenic fungi was made comparing it with the traditional plating methods. Five *A. flavus* species were identified morphologically and were in connection with aflatoxin synthesis, one from yogurt samples and 2 from both Domietti and kareish cheese samples. We founded that all strains were positive to this gene and gave one band at 800 bp. (Fig 2).

This study also focused on developing a more specific technique to facilitate *A. flavus* diagnosis as soon as possible in the early stage of contamination. Biosynthesis of mycotoxin is considered a complex process affected by the environment [49], so the use of PCR helps the prediction of the presence of aflatoxins through the detection of *A. flavus* even if it was killed. In the current, PCR has a great advantage over traditional identification methods because it is more accurate and sensitive.

The PCR technique was developed for earlier detection of *A. flavus* in solution contain *A. flavus* only or have a very small amount of *A. flavus* DNA [18], and this revealed that the use of gene-specific primers is very important for detection and differentiation of *A. flavus* from other fungi.

Conclusion and Recommendation

The results showed that high percentages of the examined samples did not comply with Egyptian standards, leading to high health risks for consumers. To counter this risk, effective safety measures need to be taken, and educational programs such as HACCP system (Hazard Analysis and Critical Control Point) should be launched for the producers of milk and dairy products.

It is recommended that the main focus should be on replacing the traditional methods Among the actual methods in use, a major focus on replacing the traditional methods as chemical preservatives by new techniques to make the consumers satisfied with less-heavily processed and preservative-free dairy products in addition to preventive and control methods used in

the manufacture of dairy products. PCR technique could be used for monitoring *A. flavus* in stored food or feed even in the case of low-level infections.

Reference

1. FAO (Food and Agriculture Organization of the United Nations) 2003. Milk and dairy products in human nutrition.
2. Barrios M T, Medina L M, Cordoba M G, Jordano R. Aflatoxin producing strains of *Asperigillus flavus* isolated from cheese. J. Food Prot. 1997; 60:192-194.
3. Garnier L, Valence F, Pawtowski A, et al. J. Diversity of spoilage fungi associated with French dairy products. Int. J. Food Microbiol. 2017a; 241: 191–197.
4. Lavoie K, Touchette M, St-Gelais D, et al. Characterization of the fungal microflora in raw milk and specialty cheeses of the province of Quebec. Dairy Sci. Technol.2012; 92: 455–468.
5. Marín P, Palmero D, Jurado M. Occurrence of moulds associated with ovine raw milk and cheeses of the Spanish region of Castilla La Mancha. Int. J. Dairy Technol. 2015; 68: 565–572.
6. Vacheyrou M, Normand A C, Guyot P, et al. Cultivable microbial communities in raw cow milk and potential transfers from stables of sixteen French farms. Int. J. Food Microbiol. 2011; 146, 253–262.
7. Sengum I, Yaman D, Gonul S. Mycotoxins and mould contamination in cheese. World Mycotoxicology J.2008; 3:291-298.
8. Khalifa M I, Al-Ashmawy M A, Abdel-Khalik A, et al. Mycological evaluation of serving some dairy products with specific reference to mycotoxins production in Azhar University Student Hostels. World J of Dairy and Food Sciences. 2013; 8:165-170.
9. Pitt J I and Hocking A D. Fungi and food spoilage. Second ed. Gaithersburg. 1999: Aspen Publishers.
10. Cary J W, Klich M A, Beltz S B. Characterization aflatoxin producing fungi outside of *Aspergillus* section Flavi. Mycologia. 2005; 97:425–432.
11. Frisvad J C, Skouboe P, Samson R A. Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B1, sterigmatocystin and 3-Omethylsterigmatocystin, *Aspergillus rambellii* sp. nov. Systematic and Applied Microbiology. 2005; 28:442–453.
12. Garnier l, Valence F, Mounier J. Diversity and control of spoilage fungi in dairy product: An update. Microorganisms. 2017b; 5(3): 42.
13. Yu J, Ahmedna M, Goktepe I. Effects of processing methods and extraction solvents on concentration and antioxidant activity of peanut skin phenolics. Food Chemistry. 2004; 90:199–206.
14. Geisen R. Molecular detection and monitoring of fungi. In: Food Mycology: a Multifaceted Approach to Fungi and Food (J. Dijksterhuis, R. A. Samson, ed.), CRC Press, Boca Raton, FL, USA. 2007; 255–278.
15. APHA (American Public Health association). Compendium of methods for the microbiological examination of foods. Third Ed. (Vanderzant, C and SPlittoesser, D. Eds) Washington DC, USA. 2003; 675-8000.
16. Kure C F, Skaar I. Mould growth on the Norwegian semi-hard cheeses Norvegia and Jarlsberg., Inter. J. Food Microbiol. 2000; 62: 133–137.
17. Collins Patricia M, Lyne J M Grange. Microbiological method, 5th Ed. Microbiology laboratory manuals. 1984; British Library Butter Worth Inco.
18. Trinh H L T, Anh D T, Thong P M, et al. A simple PCR for detection of *Aspergillus flavus* in infected food. Quality Assurance and Safety of Crops & Foods. 2015; 7 (3): 375-383.
19. Bintvihok A, Treebonmuang S S, Risakwattana K., et al. A Rapid and Sensitive Detection of Aflatoxin-producing Fungus Using an Optimized Polymerase Chain Reaction (PCR). Toxicol Res. 2016;32(1):81-87.
20. ES (Egyptian Standards), 1008-3/2005(Kareish cheese), 1008-4/ 2005 (Dometti cheese), 1008-12/2005 (Feta cheese) and 1000/2005(Yoghurt).
21. Abd El-Hameed K G. Evaluation of chemical and microbiological quality of raw goat milk in Qena Province. Assiut Vet. Med. J. 2011; 57 (129): 131-144.
22. Hameed K G A. Fungal diversity in different types of cheese and the effect of natamycin on their survival during Feta cheese manufacture and storage. J. Adv. Vet. Anim. Res. 2016; 3(3): 214-220.
23. Elbagory A M, Amal M Eid, Hammad A M, et al. Prevalence of fungi in locally produced cheese and molecular characterization of isolated toxigenic moulds. Benha Vet Med J. 2014; 27(2):9-20.
24. El Sayed M A, Hosny I M, El Kholy W I, et al. Microbiological evaluation of Egyptian white soft cheeses style. J. of American Science.2011; 7(5):517- 526.
25. Hussein F S E, El Zubeir I M, Fadelelmoula A A. Quality evaluation of imported and locally

produced processed cheese in Sudan. Jordan j of biological science. 2011; 4:231-236.
26. Aly A Salwa, Farag D E, Galal E. Effect of Gamma Irradiation on the Quality and Safety of Egyptian Karish Cheese. J. of American Science. 2012; 8(10):761-766.
27. El-Badry S, Raslan A A. Mould contamination of some Egyptian cheese. Benha Vet Med J. 2016; 30(2):28-33.
28. El-Malt L M, Abdel Hameed K G, Mohammed A S. Microbiological evaluation of yoghurt products in Qena city, Egypt Vet World. 2013;7:400- 404.
29. El-Ansary M A. Assessment of Microbiological Quality of Yoghurt Sold in El-Behera Governorate. Alexandria J of Vet Sciences. 2014; 43: 52-57.
30. Jay J M. Modern food microbiology (4th Ed.). New York, USA. Chapman and Hall. 1992;1–701.
31. Godic Torkar K, Vengus A. The presence of yeasts, moulds and aflatoxin M1 in raw milk and cheese in Slovenia. Food Control. 2008;19:570-577.
32. EL-Sharoud W, M Belloch C, Peris. D, Querol A. Molecular Identification of Yeasts Associated with Traditional Egyptian Dairy Products., J of Food Science. 2009; 74(7): M341-6.
33. Ahmad I, Gulzar M, Shahzad F. Yaqub M, et al. Quality assessment of yoghurt produced at large (Industrial) and small scale. J. Anim. Plant Sci. 2013; 23(1):58-61.
34. Bokulich N A, Mills D A. Facility-specific "house" microbiome drives microbial landscape of artisan cheese making plants. Appl. Environ. Microbiol.2013; 79, 5214–5223.
35. Schön K, Schornsteiner E, Dzieciol M, et al. Microbial communities in dairy processing environment floor-drains are dominated by product-associated bacteria and yeasts. Food Control. 2016; 70: 210–215.
36. Beletsiotis E, Guikas D, Kalantzi K. Incorporation of microbiological and molecular methods in HACCP monitoring scheme of molds and yeasts in a Greek dairy plant: A case study. Proc. Food Sci.2011; 1,1051–1059.
37. Embaby El-Sayed M, Awni N M, Abdel-Galil M M, et al. Distribution of Fungi and Mycotoxins Associated some Foods. Middle East J of Applied Science. 2015; 5 (3): 734-741.
38. Khalifa E, Nossair M. Comparative Mycological Assay on Prevalence of Yeasts, Molds and Aflatoxin M1 (AFM1) in Some Fermented Milk Products in Alexandria, Egypt. Life Science J. 2016; 13(7):20-29.
39. Pitt J I, Hocking A D. Fungi and Food Spoilage, 3rd ed.; Springer Science & Business Media: New York, NY, USA. 2009:1–2, ISBN 978-1-4615-6391-4.
40. Minervini F, Montagna M T, Spilotros G, et al. Survey on mycoflora of cow and buffalo dairy products from Southern Italy. International J of Food Microbiology.2001; 69: 141-146.
41. Nielsen M S, Frisvad J C, Nielsen P V. Protection by fungal starter against growth and secondary metabolite production of fungal spoilers of cheese. International Food Microbiology. 1998; 42: 91-99.
42. Chen C Y, Li W J, Peng K Y. Determination of aflatoxin M1 in milk and milk powder using high-flow solid-phase extraction and liquid chromatography-tandem mass spectrometry. J. Agricultural and Food Chemistry. 2005; 53(22): 8474-8480.
43. Hampikyan H, Bingol E B, Cetin O, Colak H. Determination of aflatoxin M1 levels in Turkish white, kasharandtulum cheeses. J of Food, Agriculture & Environment. 2010; 8(1): 13-15.
44. Akkaya L E, Birdane Y O, Oguz H, Cemek M U. Occurrence of aflatoxin M~ 1 in yoghurt samples from Afyonkarahisar, Turkey. Bulletin-Veterinary Institute in Pulawy. 2006; 50(4): 517.
45. Colak H. Determination of Aflatoxin M1 Levels in Turkish White and Kashar Cheeses Made of Experimentally Contaminated Raw Milk Journal of Food and Drug Analysis. 2007;15(2):163-168.
46. Lin L C, Liu F M, Fu Y M, et al. Survey of Aflatoxin M sub (1) Contamination of Dairy Products in Taiwan. J of Food and Drug Analysis.2004; 12(2): 154-160.
47. Barjesteh M H, Azizi I G, Noshfar E. Occurrence of aflatoxin M1 in pasteurized and local yogurt in Mazandaran province (northern Iran) using ELISA. Global Veterinaria.2.1.; 4(5): 459-462.
48. Shapira R, Pasti N, Fyal O, et al. Detection of aflatoxigenic moulds in grains by PCR. Appl. Environ. Microbiol.1996; 62:3270-3273.
49. Tran-Dinh N, Kennedy I., Bui T, et al. Survey of Vietnamese peanuts, corn and soil for the presence of *Aspergillus flavus* and *Aspergillus parasiticus*. Mycopathologia.2009; 168: 257-268.

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