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Mycological and Mycotoxins Analysis of Kareish and Soft Cheese in Assiut, Egypt

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Abstract During the present study 80 samples of kareish (semi-soft cheese with 20% fats) and soft cheese (40 % fats) were collected Assiut City during June to December 2015. The fungal content of these samples was evaluated using Dichloran Rose Bengal Chloramphenicol (DRBC) and Yeast Extract Malt extract (YM) agar media. The fungal count per sample ranged from 7 colonies/g in soft cheese to 44800 colonies/g in kareish cheese. the total number of fungal species in kareish cheese was slightly lower on DRBC than on YM (24 and 29 species respectively). The number of fungal species per sample fluctuated between 1-8 species with the highest being recovered from kareish cheese. *Aspergillus* was the most prevalent genus contaminating 45 -85 % of the samples with the highest being recovered from Kareish Cheese. *Penicillium* came next contaminating 20 - 47.5% of samples with white soft cheese being the most affected product. *Candida, Clavispora, Klyveromyces* and *Pichia* contaminated 2.5-65 % of samples and the highest incidence was that of Clavispora in kareish cheese. *Aspergillus niger, A. fumigatus, A. flavus, Penicillium chrysogenum, P. aurantiogriseum* and *P. brevicompactum* were the commonest species in cheese samples. Some yeast fungi were identified by sequencing of rRNA gene. Among the 19 species of yeasts *Candida tropicalis, Clavispora lusitaniae, Pichia kudriavzevii, Pichia membranifaciens* and *kluyveromyces lactis* were the most common. Testing the natural occurrence of aflatoxins revealed that AFM1 contaminated 55% and 50 % of kareish and soft cheese respectively whereas AFM2 was found only in 10 % of kareish cheese samples.

Keywords: *kareish, soft cheese, aflatoxin M1 and M2, mould and yeast fungi*

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1. Introduction

A wide variety of milk products including cheese, yoghurt, butter and ice cream are manufactured from milk of cow, buffaloes, goats, sheep and camels [1]. Cheese is made from fermented raw milk and is consumed as one of the highly nutritious dairy products in several countries. It is a good source of proteins, fats, vitamins and minerals such as calcium, magnesium and phosphorus [2].

Kareish cheese is a local type of fresh cheese in Egypt. It is preferred by Egyptian consumers due to its high protein content and low price [3]. Kareish cheese is made from skim cow or buffalo's milk which is extracted directly into special earthenware pots and kept without disturbance to allow the fat to rise to the surface forming a cream layer. The cream layer is then removed, and the curd is poured onto a mat which is tied and hung for few days to allow the drainage of the whey. The cheese is then cut into suitable pieces with the addition of some salt. The cheese is left for few hours in the mat till whey no longer drains out, then it is ready to be consumed as fresh soft cheese containing about 20% fats. The second type of cheese is produced by some dairy companies in Egypt with 40% fat content. Because milk and milk products are rich in moisture and several nutrients they are subjected to microbial contaminated which grow and multiply causing spoilage and economic losses to the dairy industry [4]. The contamination of milk products with different types of fungi particularly of species of Aspergillus, Fusarium and Penicillium constitute a public health hazard as these fungi are known to produce mycotoxins that are injurious to human health [5]. Some of the common yeasts related to dairy products are: *Debaryomyces hansenii*, *Galactomyces geotrichum*, *Yarrowia lipolytica*, *Kluyveromyce marxianus*, *K. lactis var. lactis*, and *Saccharomyces cerevisiae* [6].

Aflatoxins are natural compounds produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins include B1, B2, G1, and G2. B1 is usually found at high concentration in contaminated food and feedstuffs [7]. Aflatoxins M1 and M2 are derivatives of B1 and B2, which are excreted in the milk of animals [8].

This investigation was carried out to study the prevalence and density of filamentous and yeast fungi in two types of cheese and to evaluate the natural occurrence of mycotoxins in these products.

2. Materials and Methods

2.1. Collection of Samples

Sample of kareish and soft cheese (40 samples for each) were randomly collected from different local markets in Assiut City, Egypt during the period from June to December 2015. Samples were kept at -20°C till mycological and toxicological analysis.

2.2. Mycological Analysis

The dilution plate technique was employed to isolate fungi contaminating kareish and soft cheese using dichloran rosebengal chloramphenicol (DRBC) and yeast extract-malt extract (YM) agar [9]. Cultures were incubated at 28±2°C for 7-10 days after which the developing fungal colonies were counted, identified and preserved.

2.3. Identification of Fungal Isolates

The following references were used for phenotypic identification [9,10,11,12]. Molecular characterization of some yeast isolates was done with the help of Solgent Company, Daejeon South Korea [13].

2.4. Extraction and Detection of Mycotoxins

This part was performed with the help of food analysis center, Faculty of Veterinary Medicine, Benha University according to the following protocol:

2.4.1 Ten grams of each cheese sample was blended with 100 ml extraction solvent: acetone: water (85:15, v/v) for 30 min. Then the mixture was filtered through Whatman No. 1 filter paper. After filtration, the extract (5 ml) was diluted with water (75 ml).

2.4.2 High performance liquid chromatography (HPLC) used for aflatoxin determination was an Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254nm wavelength. Also, auto sampler (model G1329A VP-ODS) and Shim pack (150× 4.6 mm) column (Shimadzu, Kyoto, Japan) were used.

2.4.3 The stock standard solutions of AFB1, AFB2, AFM1 and AFM2 were prepared by dissolving the solid standard in benzene: acetonitrile (98:2, v/v).

2.4.4 The European Commission was used as for guidelines and criteria to assess the method validation. Selectivity was determined from Retention time, ion ratios, and identification-points (IP) for each analyte. Calibration standards were prepared by combining standard solutions into the solvent and blank matrix extracts (matrix-matching) to yield the desired concentrations in the range of 10-500 µg/L for each analyte.

3. Results and Discussion

3.1. Fungi Isolated from Kareish Cheese

Thirty-one species belonging to 21 genera were isolated from kareish cheese samples on both DRBC (23 species) and YM (27 species)

3.1.1. On DRBC medium

With reference to Table 1 and Table 2, the total number of fungal colonies per sample ranged from 1520-44800 colonies / g of kareish cheese. The number of species per sample ranged from 2- 8 species with the richest samples being No. 81 and No. 93.

The most common genera were *Aspergillus*, *Clavispora* and *Pichia* being isolated from 85%, 60% and 50% of samples respectively. The percentage count of *Clavispora* (26.28%) was markedly higher than that of *Aspergillus* and *Pichia* (7.10%, and 13.27% of the gross total population of fungi respectively). *Clavispora lusitanie* was the only representative species of its genus.

Pichia was represented by 4 species of which *P. kudriavzevii* was of moderate incidence (32.5% of total samples matching 10.7% of total count of fungi). *P. anomala, P. cactophila and P. membranifacenss* occurred in low incidence. *Candida tropicalis, Galactomyces candida* and *Magnsiomyces* sp. were isolated from 40%, 40% and 27.5% of kareish cheese samples (moderate incidence) accounting for 13.71%, 14.1% and 8.18% of total fungal population respectively. Also, *Kluyveromyces* contaminated 30% of kareish cheese samples accounting for 8.55%of total fungal population. *Kluyveromyces* was represented by 2 species namely *K. lactis* and *K. marxianus* (25% and 7.5% of cheese samples respectively).

3.1.2. OnYM Medium

With reference to Table 1 & Table 2, the total number of fungal colonies per sample ranged from 1680-43200 colonies / g. The number of species ranged from (2 - 6 species / sample). *Aspergillus, Pichia* and *Clavispora* were the most common genera being isolated from 67.5%, 52.5% and 65% of kareish cheese samples respectively. The percentage counts of *Pichia* and *Clavispora* (22.66% and 23.27% respectively) were much higher than that of *Aspergillus* 5.19% of the gross total population of fungi. *Aspergillus* was represented by 4 species of which *A. flavus* and *A. niger* occurred in 30% and 42.5% of samples matching 2.88% and 2.07% of total count of fungi respectively. Four species of Pichia were recovered of which *P. kudriavzevii* occurred in moderate incidence (27.5% of the total samples) matching 14.35% of total count of fungi. Each of *Clavispora* and *Galactomyces* was represented by one species (*C. lusitanii* and *Galactomyces candida* which occurred in high and moderate incidences 65% and 37.5% of samples matching 22.66 and 11.14% of total count of fungi respectively.

Most of the moulds that contaminate kareish cheese in this work especially those belonging to *Aspergillus, Penicillium, Cladosporium, Mucor* and *Fusarium* were also isolated by several investigators [14,15,16,17,18]. Similarly the major yeasts contaminating kareish cheese

especially *Candida lipolotica* (= *Yarowiiai lipolotica*), *C. parapsiliopsis C. catenulata, C. tropicalis* and *C. zeylanoides, Kluyveromyces lactis* and *K. Marxianus, Rhodotorula* sp., *Pichia* sp., *Cryptococcus* sp. and *Galactomyces candida* were recovered from similar samples [14,15,16,18,19,20].

3.1.3. Phylogeny of Yeasts Isolated from Kareish Cheese

With reference to Fig. (1), the phylogenic tree covered eight different yeast species. Identification of each species was done on the basis of its high similarity with reference strains in the Gene Bank. Two species of *Pichia* were identified, namely *Pichia kudriavzevii* and *Pichia anomala*. The remaining species included *Galactomyces candidum* (anamorph=*Geotrichum candidum*), *Candida tropicalis*, *Clavispora lusitaniae*, *Trichosporon asahii*, *Lecythophora* sp. and *Kluyveromyces lactis*.

3.2. Fungi Isolated from Soft Cheese

A total of 38 species belonging to 22 genera were recovered from the 40 soft cheese samples using DRBC (17 genera and 29 species) and YM (17 genera and 30 species) as shown in Table 1 & Table 2. Three out of the 40 samples were free of fungal propagules on both DRBC and YM media.

3.2.1. On DRBC Medium

The total number of fungal colonies per sample ranged from 6.7-3153.02 colonies / g of soft cheese (Table 1 & Table 2). Seven (17.5%) of white cheese samples were completely free of fungi. The number of species ranged from (1 - 6 species / sample) .

Penicillium was isolated from 50% samples matching 29.5% of total population of fungi. P. chrysogenum occurred in moderate incidence contaminating 30% of white cheese samples accounting for 8.43% of the total fungal population. *Aspergillus* and *Clavispora* occurred in modrate incidence contaminating 47% and 37.5% of white cheese samples respectively. The precentage count of *Clavispora* markedly exceeded that of Aspergillus (33.3% and 9.21%respectively). *Clavispora* was represented only by one species (*Clavispora lusitanii*). *Aspergillus* was represented by 6 species of which *A. niger* occurred in 25% of samples accounting for 2.19% of the total fungal population.

3.2.2. On YM Medium

In contaminated samples the total number of fungal colonies per sample ranged from 6.6-6120 colonies/g (Table 1 & Table 2). Four samples (10 %) were completely free of fungi. The number of species ranged from (1 - 8 species / sample). *Aspergillus, Penicillium, Clavispora* and *Pichia* occurred in modrate incidence and were isolated from 45%, 47.5%, 35% and 17.5%of white cheese samples respectively. The count of *Clavispora* (27.3%) markedly exceeded that of *Aspergillus, Penicillium* and *Pichia7*.80%, 18.24% and 9.07% of the gross total fungal population respectively.

Among the 5 species of *Aspergillus, A. fumigatus* was contaminating 32.5% of soft cheese samples accounting for 3.78% of the total fungal population. Eight species of Penicillium were identified from which *P. chrysogenum* appeared in 30% of samples representing 7.35% of the total fungal count. *Clavispora lusitanii, Pichia kudriavzvii, P. parapsiliopses* and *P. membranifacens* were the representative species of corresponding genera.

A lot of Egyptian investigators analyzed soft cheese for yeast and mould contamination among them Ibrahim et al. (2015) [21], Sayed et al. (2011) [22] who reported that yeasts and moulds contaminated 100% of tested white soft cheese samples while, ELbagory et al. (2014) [23] and Hamms (2014) [16] found yeasts in 77.1% and 25% and molds in 94.3% and 20% of samples respectively. Differences in the count of fungal propagules may be due to the geographical location of producing companies, duration of storage and the hygienic status of persons dealing with these products. Lund et al. (1995) analyzed soft cheese from Denmark and isolated *Penicillium auarantiogriseum* and *P. chrysogenum* [24]. Johnson (2001) isolated *Geotrichum candidum, Kluyveromyces marxians, Pichia* sp and *Candida* sp. from cheese [25]. Torkar and Teger (2006) from Slovenia reported that 60% of tested cheese samples were contaminated with yeasts and moulds which included *Aspergillus* sp., *Penicillium* sp., *Geotrichum candidum* and *Moniliella spathulate* [26]. Lavoie et al. (2012) analyzed cheese samples from Quebec and isolated *Candida* (*C. catenulata, C. parapsiliopsis, C. parragosa, C. tropicalis, C. zeylanoides*), *Cryptococcus curvatus, Pichia* (*P. kudriavzevii, P. mempranifacens), Rhodotrola mucilaginosa, Trichosporon asahii, Trichosporon jirovece, Kluyveromyces marxianus, A. fumigatus, C. cladosporioides, Erotium* sp and *Mucor circinelloides* [27]. Allaraj et al. (2013) [28] and Khalifa et al. (2013) [5] isolated *Aspergillus flavus, A. niger, Penicillium chrysogenum, Mucor* sp., *Cladosporium* sp., and *Fusarium* sp. from cheese while Banjara et al. (2015) obtained some yeasts including (*Geotrichum candidum, Kluyveromyces lactis, Pichia kudriavzevii, C.parapsiliopsis*) [29]. Also *Aspergillus* sp., *Penicillium* sp., *Mucour* sp., *Cladosporium* sp., *Rhizopus* sp., *Scopulariopsis* sp., *Candida* sp., *Rhodotorula* sp were isolated from cheese by ELbagory et al. (2014) [23] and Hameed (2016) [17]. While Chipilev et al. (2016) isolated these moulds from cheese in addition to *Candida* sp. and *Rhodotrola* sp. at percentages of 70% for molds and 63.3% for yeasts [30].

It was very interesting to observe that the total fungal load contaminating kareish cheese was much higher than that in soft cheese (42 folds) as shown on DRBC medium. Nearly the same observation was recorded in case of YM medium whereas the gross fungal population obtained from kareish cheese was 26.3 folds that of soft cheese. A plausible explanation of the reduced number of fungal propagules in soft cheese is the addition of relatively high amount of NaCl salt which reduce the vegetative growth of several molds and yeasts. Also, thermal treatment of milk during pasteurization often contributes to eliminate many of the contaminating fungi.

The heavy contamination of kareish cheese by molds an and yeasts is often attributed to exposure of milk to air mycobiota during processing, transport and marketing of this kind of cheese. Most of kareish cheese is made by dairy farmers, who often don’t follow the correct higyenic measures in their work.

Table 1. Counts (cfu/g) and number of species of fungi isolated from kareish and soft cheese on DRBC and YM media

Kareish cheese					Soft cheese				
	DRBC		YM			DRBC		YM	
Sample No.	**Fungal count**	**No. of species**	**Fungal count**	**No. of species**	**Sample No.**	**Fungal count**	**No. of species**	**Fungal count**	**No. of species**
1	3760	8	2820	4	**41**	520	3	880	3
2	1520	4	1680	4	**42**	0	0	4280	5
3	7600	5	6980	5	**43**	100	2	40	1
4	3500	5	3440	5	**44**	240	3	380	3
5	8160	6	7680	5	**45**	520	4	940	3
6	3940	4	3540	4	**46**	1960	3	6120	6
7	8680	3	8560	3	**47**	200	2	40	1
8	9360	5	8920	4	**48**	360	3	80	1
9	9520	5	9080	4	**49**	360	3	300	3
10	9680	4	9000	3	**50**	860	5	846.6	5
11	6880	5	8760	3	**51**	1093	4	779.9	2
12	9120	4	10360	6	**52**	220	2	213.3	3
13	9000	8	7520	3	**53**	0	0	0	0
14	20480	6	15360	2	**54**	700	2	100	2
15	15440	4	17200	2	**55**	2160	5	2440	5
16	9200	3	13200	3	**56**	1400	6	1900	5
17	18960	6	18480	5	**57**	0	0	93.3	1
18	18880	5	19200	3	**58**	427	5	453.3	2
19	20000	2	23120	4	**59**	367	2	619.9	2
20	22560	4	24240	4	**60**	0	0	60	2
21	18240	3	18480	3	**61**	0	0	0	0
22	18320	3	19760	3	**62**	173	3	333.3	4
23	15040	3	15120	5	**63**	213	2	126.7	2
24	39040	5	43200	3	**64**	1680	6	1480	5
25	40960	5	37760	5	**65**	1740	4	2760	8
26	30880	4	28320	3	**66**	7	1	186.7	2
27	32800	4	23680	5	**67**	0	0	0	0
28	11360	4	16960	4	**68**	33.3	3	100	4
29	32160	6	33440	6	**69**	60	2	93.3	3
30	37920	2	32480	6	**70**	0	0	110	5
31	24160	4	24640	3	**71**	3153	2	2499.8	3
32	27680	6	23200	5	**72**	10	1	10	1
33	22720	4	23360	5	**73**	13.3	2	13.3	2
34	41600	6	30080	4	**74**	147	4	40	2
35	44800	3	33920	2	**75**	13.3	2	6.6	1
36	34880	3	35840	5	**76**	6.7	1	0	0
37	32640	3	30560	3	**77**	27	2	60	2
38	9760	4	8160	4	**78**	40	2	93.3	3
39	24480	5	23200	5	**79**	27	2	73.3	4
40	37600	4	32480	4	**80**	67	2	106.7	1

Table 2. Total counts (TC as colonies/g in all samples) and frequencies of occurrence (F) of fungal genera and species isolated from kareish and soft cheese

Genera and Species	Kareish cheese						Soft cheese					
	DRBC			YM			DRBC			YM		
	%TC	F&OR	%F	%TC	F&OR	%F	%TC	F&OR	%F	%TC	F&OR	%F
Alternaria alternata	0	0	0	0	0	0	0	0	0	0.42	1 L	2.5
Aspergillus	7.10	34 H	85	5.19	27 H	67.5	9.21	19 M	47.5	7.80	18 M	45
A. carneus	0	0	0	0	0	0	0.21	2 L	5	0	0	0
A. flavus	2.9	20 M	50	3	14 M	35	2.22	8 L	17.5	1.84	5 L	12.5
A. fumigatus	1.31	10 M	25	0.05	3 L	7.5	3.42	9 L	22.5	3.78	13 M	32.5
A. nidulans	0.05	1 L	2.5	0.04	1 L	2.5	0.07	1 L	2.5	0.02	1 L	2.5
A. niger	2.83	16 M	40	2.07	17 M	42.5	2.19	10 M	25	1.67	7 L	17.5
A. sulphureus	0	0	0	0	0	0	1.16	1 L	2.5	0	0	0
A. sydowii	0	0	0	0	0	0	0	0	0	0.02	1 L	2.5
A. terreus	0	0	0	0.07	1 L	2.5	0	0	0	0.49	1 L	2.5
Chaetomium globosum	0.04	1 L	2.5	0	0	0	0.11	1 L	2.5	0	0	0
C. cladosporioides	0.01	1 L	2.5	0	0	0	0.07	2 L	5	0.14	1 L	2.5
Fusarium	0.03	1 L	2.5	0.04	1 L	2.5	0.04	1 L	2.5	0	0	0
F . oxysporum	0	0	0	0.04	1 L	2.5	0	0	0	0	0	0
F . Solani	0	0	0	0	0	0	0.04	1 L	2.5	0	0	0
F . Verticillioides	0.03	1 L	2.5	0	0	0	0	0	0	0	0	0
Mucor himalis	0	0	0	0.04	1 L	2.5	0	0	0	0.56	1 L	2.5
Paeciiomyces sp.	0	0	0	0	0	0	0	0	0	0.56	1 L	2.5
Penicillium	3.14	9 L	22.5	3.85	8	20	29.51	20 H	50	18.24	19 M	47.5
P . aurantiogriseum	0.61	5 L	7.5	0.99	4 L	10	16.9	4 L	10	8.32	3 L	7.5
P. brevicompactum	0.24	1 L	2.5	0.25	1 L	2.5	0.42	1 L	2.5	0.02	1 L	2.5
P. chrysogenum	1.81	9 L	22.5	2.1	6 L	15	8.43	12 M	30	7.35	12 M	30
P. citrinum	0	0	0	0	0	0	2.15	4 L	10	0.33	1 L	2.5
P . duclauxii	0.04	1 L	2.5	0	0	0	0	0	0	0	0	0
P. griseofulvum	0	0	0	0	0	0	0.12	2 L	5	0.8	2 L	5
P . oxalicum	0	0	0	0	0	0	0	0	0	0.23	1 L	2.5
P. pinophilum	0.44	1 L	2.5	0.51	1 L	2.5	0	0	0	0.07	1 L	2.5
P. purprogenum	0	0	0	0	0	0	1.48	1 L	2.5	1.12	1 L	2.5
R . stolonifer	0	0	0	0.04	1 L	2.5	0	0	0	0.42	2 L	5
Scopulariopsis	0	0	0	0.02	1 L	2.5	0.04	1 L	2.5	0.22	3 L	7.5
Scopulariopsis bromptii	0	0	0	0.02	1 L	2.5	0.04	1 L	2.5	0.22	3 L	7.5
Setosphaeria rostrata	0	0	0	0	0	0	0.64	1 L	2.5	0	0	0
Synspholastrum racemosum	0	0	0	0.07	1 L	2.5	0	0	0	0	0	0
Black yeasts	0	0	0	0.16	3 L	7.5	0	0	0	0	0	0
Candida	13.71	16 M	40	8.2	10 L	25	3.70	3 L	7.5	13.64	6 L	15
C.parapsiliopsis	0	0	0	0	0	0	0.21	1 L	2.5	1.64	3 L	7.5
C. tropicalis	13.71	16 M	40	8.2	10 L	25	3.49	2 L	5	12	4 L	10
Clavispora lusitaniae	26.28	24 H	60	23.27	26 H	65	33.3	15 M	37.5	27.3	14 M	35
Galactomyces candidas	14.1	16 M	40	11.14	15M	37.5	2.96	1 L	2.5	3.7	1 L	2.5
Kluyveromyces	8.55	12 M	30	8.84	10L	25	2.65	1 L	2.5	6.77	5 L	12.5
Kluyveromyces lactis	8.22	10 M	25	8.36	9 L	22.5	2.65	1 L	2.5	6.77	5 L	12.5
Kluyveromyces marxians	0.34	3 L	7.5	0.47	1 L	2.5	0	0	0	0	0	0
***Lecythophora* sp.**	1.77	3 L	7.5	2.13	4 L	10	0	0	0	0	0	0
Magnisiomyces capitatus	8.18	1 1 M	27.5	5.95	7 L	17.5	0	0	0	2.09	2 L	5
Moniliella spathulata	0	0	0	0	0	0	1.87	1 L	2.5	2.51	2 L	5
Pichia	13.27	20 H	50	22.66	21 H	52.5	11.54	10 L	25	9.07	10 M	25
P. anomela	1.61	6 L	15	2.13	5 L	12.5	0	0	0	0	0	0
P. cactophila	1.95	6 L	15	7.94	7 L	17.5	0.21	1 L	2.5	0	0	0
P. kudriavzevii	10.7	13 M	32.5	14.35	11M	27.5	12.17	6 L	10	7.88	6 L	15
P.manshurica	0	0	0	0	0	0	1.87	1 L	2.5	0	0	0
P. membranifaciens	2.64	4 L	10	1.55	2 L	5	0.67	4 L	10	1.12	4 L	10
P. parapsiliopses	0	0	0	0	0	0	0	0	0	0.07	1 L	2.5
Rhodotrola mucilaginosa	0	0	0	0.53	1 L	2.5	0.28	1 L	2.5	0	0	0
Trichosporon	0.11	1 L	2.5	0.76	2 L	5	0	0	0	0.42	2 L	5
Trichosporon asahii	0.11	1 L	2.5	0.76	2 L	5	0	0	0	0	0	00
Trichosporon insectroum	0	0	0	0	0	0	0	0	0	0.42	2 L	5
Yarowiilipolotica	0	0	0	3.74	2 L	5	0.53	1 L	2.5	6.68	5 L	12.5
Total count	**793280**			**753780**			18895.99			28659.28		
NO of genera 33	**14**			**18+1**			17			17		
NO of species 66(63+3)+1	**23+1+1**			**27+1+2**			29+2			30+1		

OR = Occurrence Remarks: H = High Occurrence, 10-20 cases; M = Moderate Occurrence, 5-9 cases, L = Low Occurrence, 1- 4 cases.

3.2.3. Phylogeny of Yeasts Isolated from Soft Cheese

Performing 18S rDNA sequencing allowed the molecular identification of 10 different yeast species (Figure 2). Identification of each species was based on its close relationship with reference strains in the Gene bank. Three species of Pichia were identified, namely *P. membranifaciens*, *P. manshurica* and *P. cactophila*. Also, two species of *Candida* were identified (*C. tropicalies* and *C. parapsiliosis*). The remaining species including *Yarrowia lipolytica*, *Rhodotorula mucilaginosa*, *Clavispora lusitanii*, *Trichosporon insectorum* and *Moniliella spathulata*.

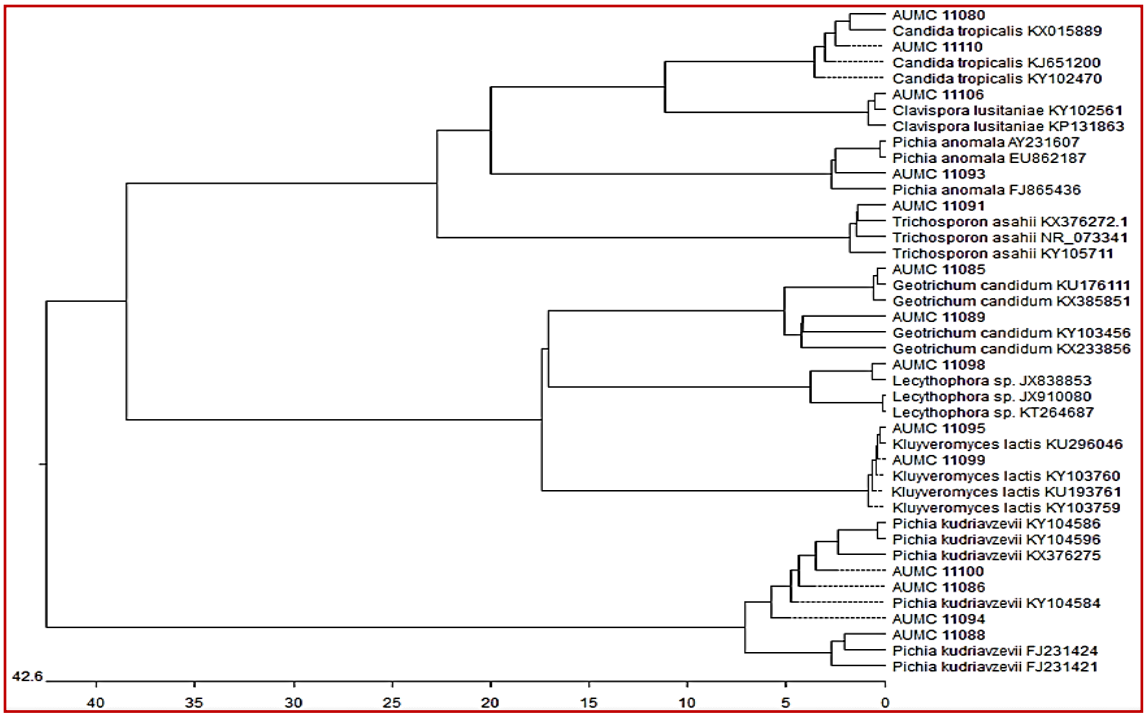

Figure 1. Phylogenetic tree for fungal species isolated from kareish cheese (given AUMC numbers). Reference strains of corresponding fungi are involved in the tree (given CBS, ATCC or AUMC numbers)

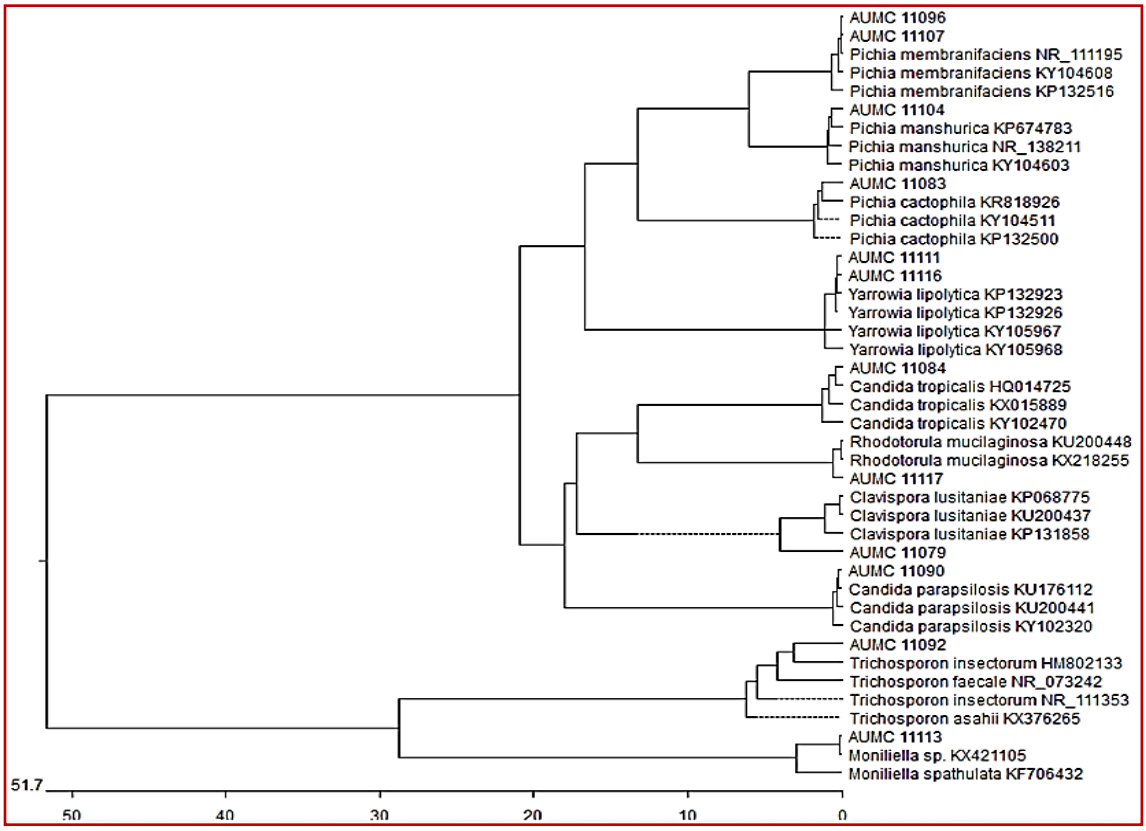

Figure 2. Phylogenetic tree for fungal species isolated from white cheese (given AUMC numbers). Reference strains of corresponding fungi are involved in the tree (given CBS, ATCC or AUMC numbers)

3.3. Aflatoxins Contaminating Kareish and Soft Cheese

The presence of Aflatoxins M1, M2, B1 and B2 in 30 collected samples of kareish (20 samples) and soft cheese (10) was tested using HPLC. Sixteen samples (53.33%) were contaminated with Aflatoxin M1 (5 and 11 samples of soft cheese and kareish respectively). Aflatoxin M2 was detected only in two samples of kareish cheese as shown in Table 3.

Table 3. Natural occurrence of Aflatoxins in soft cheese samples.

No. of tested Samples	No. of positive Samples	Aflatoxins (ug/Kg) "ppb"			
		M1	M2	B1	B2
10 (Soft cheese)	5	1.337	-	-	-
		0.670	-	-	-
		0.220	-	-	-
		0.141	-	-	-
		1.425	-	-	-
20 (Kareish cheese)	11	1.463	-	-	-
		0.245	-	-	-
		1.219	0.073	-	-
		1.030	-	-	-
		0.606	-	-	-
		0.360	-	-	-
		0.172	-	-	-
		0.250	-	-	-
		1.980	0.155	-	-
		1.827	-	-	-
		0.941	-	-	-

The amount of Aflatoxins M1 in positive samples ranged from (0.141-1.980 ug/kg). The highest amount of Aflatoxins M1 was present in kareish cheese while the lowest was in soft cheese samples. All of tested samples were free from Aflatoxins B1 and B2. Levels of AFM1 contamination in kareish cheese ranged from 0.172 to1.980 ug/Kg). Levels of AFM2 in the two positive samples of Kareish were 0.073 and 0.155ug/Kg. In case of soft cheese, AFM1 was detected in 5 out of 10 samples (50 %), and the contamination level ranged from 0.141 to 1.337 ug/Kg. Contamination of cheese with AFM1may be due to the presence of this toxin in the milk of animals that are fed with aflatoxin B1(AFB1) containing feed [31] or dried milk used in manufacturing cheese [32].

In Egypt, El-Sherief (2000) [33] examined 15 kareish cheese collected from Assiut city, Egyptand found that 2 samples out of 15 kareish cheese tested samples (13.3%) were contaminated with AFM1 with range 1.75- 3.47 mg/ Kg. Gab-Alla et al. (2005) reported that AFM1, AFB1 and mix of AFM1 and AFB1 contaminated 12 samples out of 50 kareish cheese tested samples (24%) [34]. El-Diasty and Salem (2007) analyzed 20 samples of kareish cheese and found that 20% of samples were contaminated with AFM1 at a range of 5000 to 3500 ppt [14]. Awad et al. (2014) tested 25 samples of kareish cheese and reported that the mean value of AFM1 was 3600 ppt with contamination percentage of 48% [35]. El-kest et al. (2015) found that all of the 20 samples of kareish cheese collected from Cairo were contaminated with AFM1 and the average concentration was 140.8 - 528 ppb [36]. In Mansura Governorate, Egypt Younis et al. (2016) evaluated the AFM1 in kareish cheese and observed that 60 % of samples contained the toxin at a concentration of 0.088 ± 0.024 ppb [37]. El-Sherief (2000) examined 15 soft cheese samples and found that AFM1contaminate (80%) with the range level of contamination (3.40- 13.75 mg/Kg) [33]. Awad et al. (2014) examined 25 samples of soft cheese for AFM1 contamination and found that mean value was 6700 ppt respectively with contamination percentage 32% of soft cheese [35]. In Turkey, Var and Kabak (2009) [38], Tekinşen and Uçar (2008) [39] and Ertas et al. (2011) [40] analyzed 20, 100 and 72 cheese samples collected from different cities of turkey and found that 80%, 99% and 94.4% of cheese samples were contaminated with AFM1 respectively. Also, they reported that the level of AFM1contamination in tested cheese samples were 50-800ng/kg, 0-4100 ng/Kg and 0.012-0.378 mg/Kg respectively. Fallah et al. (2009) [41] and Mariko Kubo (2012) [42] from Iran examined 116 and 80 cheese samples respectively, for AFM1 contamination and found that 80.1% and 82.5% of tested cheese samples contaminated with AFM1 respectively. In Brazil, Iha et al. (2011) [43] and Jager et al. (2013) [44] analyzed cheese samples for AFM1 contamination and found that 84% and 30% of the samples were respectively contaminated with AFM1. In Kuwait, Dashti et al. (2009) [45] found that 80% of cheese samples contained AFM1 at levels of 0.024 to 0.425ng/Kg .

Nearly similar levels of AFM1 contamination of cheese were reported from Sudan (Elzupir and Elhussein, 2010) [46] and Pakistan (Iqbal et al., 2013) [47] where the levels ranged from 79.5- 389 ng/Kg and 0.091- 0.3mg/Kg respectively.

On the other hand, Khalifa et al. (2013) analyzed 130 cheese samples for AFM1 contamination and found that, all cheese samples contaminated with *Aspergillus* spp. were free from AFM1[5].

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