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ENZYMES AND TOXINS PRODUCED BY FUNGI ISOLATED FROM MILK AND MILK PRODUCTS IN ASSIUT, EGYPT.

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A total of 102 fungal strains obtained from fresh cow and buffalo milk as well as from yoghurt, kareish cheese and soft cheese were screened from their abilities to produce proteolytic and lipolytic enzymes. The tested strains comprised 52 yeasts and 50 filamentous fungi. The most active producers of both lipases and proteases were *Candida paragosa, C. zeylanoides, Cryptococcus curvatus, Fusarium oxysporum* (from baffalo milk), *Magnesiomyces capitatus, Trichosporon jirovecii* (from cow milk), *Candida tropicalis, Geotrichum candidum, Lecythophora sp., Pichia kudriavzevii, Aspergillus flavus* (from kareish cheese), *P. cactophila, T. insectorum* and *Yarrowia lipolytica* (from soft cheese), *A. niger* and *Fusarium verticillioides* (from yoghurt). Among the 50 mold strains tested for mycotoxin production in broth cultures 30 (60%) proved to be toxinogenic. Aflatoxins $B_1$and G_1 were detected in cultures of *A. flavus* (17 strains) and *A. parasiticus* (4 strains) obtained from various samples of milk and milk products. Sterigmatocystin was produced by 5 strains of *A. nidulans*. Fumonisin and zearalinone were detected in cultures of *F. verticilliodes* and *F. oxysporum,* respectively.

Key words: mycotoxin, Aflatoxin B_1, Aflatoxin G_1, sterigmatocystin, Fumonisin, lipolytic, proteolytic, yeast, filamentous fungi

INTRODUCTION

Lipolytic and proteolytic enzymes are extracellular enzymes produced by spoilage mycobiota resulting in off-flavour due to lipid and protein degradation (**Downey 1980; Conesa *et al*. 2001**), which adversely affects the quality of milk products (**Cormie 1992**) leading to economic losses and reduction of the shelf-life time of the products. Filamentous fungi belonging to various species of *Aspergillus*, *Rhizopus*, *Penicillium* and *Trichoderma* are described as important sources of lipolytic and proteolytic enzymes (**Lima *et al*. 2003; Kashmiri *et al*. 2006; Karanam and Medicherla 2008**).

The contamination of raw milk and milk products with various fungal species, especially those belonging to *Aspergillus*, *Fusarium* and *Penicillium* constitutes a public health hazard. These fungi produce different types of mycotoxins that are dangerous to human health (**Sengum *et al*. 2008; Khalifa *et al*. 2013**). Aflatoxins (B_1, B_2, G_1 & G_2) are often produced by *Aspergillus flavus* and *A. parasiticus* (**Frisvad *et al.* 2005**). Aflatoxin B_1 represents the highest degree of toxicity for humans, followed by G_1, B_2 and G_2 (**Gourama and Bullerman 1995; Sweeney and Dobson 1998**). About five billion people in developing countries are at high risk of chronic exposure to aflatoxins during consuming contaminated food (**Shephard and Sewram 2004**).

The present investigation was designed to study the potential of some fungi previously isolated from milk and milk products to produce lipolytic and proteolytic enzymes as well as mycotoxins.

MATERIALS AND METHODS

1- Collection of samples

A total of 200 samples of raw milk (cow milk and buffalo milk), yoghurt kareish, and soft cheese (40 samples for each) was collected randomly from different supermarkets, groceries, street vendors and dairy shops in Assiut Governorate during the period from October 2013 to December 2015. Samples (500 g each) were transferred immediately to the laboratory and kept in a deep freezer (-20°C) for mycological analysis.

2-Media used during the current study

a) Yeast extract, malt extract agar (YM) has the composition of (g/l): yeast extract, 3; malt extract, 3; peptone, 5; glucose, 10; agar, 20 **(Wickerham 1951)**.

b) Dichloran Rose Bengal Chloramphenicol agar (DRBC): has the composition of (g/l): Peptone, 5; Glucose, 10; KH2PO4, 1; MgSO4.7H2O, 0.5; Dichloran (0.2% in ethanol), 1ml; Rose Bengal, 0.025; Chloramphnicol, 0.1; Agar, 15.

c) Czapek's Dox Agar (Cz) has the composition of (g/l): sodium nitrate, 2; potassium chloride, 0.5; magnesium sulfate, 0.5; dipotassium hydrogen ortho-phosphate, 1; zinc sulfate, 0.01; copper sulfate, 0.005 and agar, 20.

d) Potato Dextrose agar (PDA) has the composition of (g/l): potato infusion, 200; dextrose, 20 and agar, 20.

3- Mycological analysis

The dilution plate technique recommended by **Pitt and Hocking (2009)** was employed to isolate fungi contaminating kareish and soft cheese using DRBC and YM media. Cultures were incubated at 28±2 °C for 10 days to filamentous fungi and for 3 days to yeast isolate after which the developing fungal colonies were counted, identified and preserved.

4- Identification of fungal isolates

The following references were used for phenotypic identification; **Raper and Fennell (1965)** for *Aspergillus* and its teleomorphs; **Leslie and Summerell (2006)** for *Fusarium*. Molecular characterization of some yeast isolates was done with the help of Solgent Company, Daejeon, South Korea as mentioned by **Zohri et al. (2014)**.

A total of 102 fungal strains (50 of filamentous fungi and 52 strain of yeast) obtained from (fresh cow and buffalo milk as well as from yoghurt, kareish cheese and soft cheese) were screened from their abilities to produce proteolytic and lipolytic enzymes and for mycotoxin production.

5-Microorganisms and preparation of inoculums

A 7-day-old pure cultures of *Aspergillus* strains grown on Czapek's Dox agar at 28 °C, 5-day-old cultures of *Fusarium* strains grown on PDA at 28 °C, and 3-day-old cultures of yeast species grown on YM at 28 °C were harvested in 10 % tween 80 to form a spore suspension had the concentration of 1 x 10^7 spore/ml. The spore suspensions were used for inoculation of enzyme production media and mycotoxin production medium.

6-Lipolytic activity

It was carried out on the medium described by **Ullman and Blasins (1974)**. The medium has the composition of (g/l): peptone, 10; $MgSO_4 .7H_2O$, 0.2; $CaCI_2.2H_2O$, 0.2; Tween 20, 10 ml; and agar, 15). Tween 20 was autoclaved separately and added to the sterile and cooled basal medium. The medium was dispensed aseptically into 15-cm test tubes (10 ml each). The tubes were inoculated, individually, with 50 µl spore suspension and incubated at 28 °C for 10 days. The lipolytic ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of each visible precipitate (in mm) was measured.

7-Proteolytic activity

It was employed using casein hydrolysis medium described by **Paterson and Bridge (1994).** The medium has the composition of (g/l): KH_2PO_4, 1.0; KCL, 0.5; $MgSO_47H_2O$, 0.2; $CaCI_2.2H_2O$, 0.1; glucose, 10, agar, 15 and distilled water to one liter). After autoclaving at 121 °C for 20 minutes, 25 ml of 15 % skimmed milk (sterilized at 115 ^{o}C for 10 min) was added to the medium and the medium was poured into 15-cm test tubes (~10 ml each). The tubes were inoculated, individually, with 50 µl spore suspension and incubated at 28 °C for 7 days. After incubation, complete degradation of milk protein appeared as clear depth in the tube. The clear depth below the colony was measured (in mm).

8-Mycotoxin production by fungal strains

Fifty strains related to six species, namely *Aspergillus flavus* (20 strains), *A. niger* (10), and *A. nidulans* (6), *A. parasiticus* (4), *Fusarium oxysporum* (4) and *F. verticillioides* (6) were screened for their abilities to produce mycotoxins (Table 1). Potato dextrose broth medium was dispensed in 250 ml-Erlenmeyer conical flasks (50 ml each). The flasks were, individually, inoculated with 1 ml spore suspension and incubated for 10 days at 28 °C in a static condition.

Mycotoxins were extracted from fungal cultures by chloroform as described by **Refaie (2013).**

TLC analysis was carried out on commercialized, ready-to use silica gel plates (20 ×20) cm developed in chloroform: methanol (96:4) solvent. The chromatographic properties of the fungal crude extracts were compared with appropriate reference standards (Aflatoxins, Sterigmatocystin, Zearalinone and Fumonisin) as described by **Refaie (2013).**

RESULTS AND DISCUSSION

1-Lipolytic and proteolytic activities of filamentous and yeast fungi

Lipolytic and proteolytic activities of one hundred and two strains; 50 of filamentous fungi belonging to seven species and 52 of yeasts related to 27 species were evaluated.

Lipolytic activity

Forty-one strains representing 82 % of total filamentous fungi showed positive results from which 10, 15 and 16 strains possessed high, moderate and low abilities of lipases production respectively. All strains of *A. niger* (10 strains) recorded high ability of lipase production (Table 1). *Aspergillus flavus* (8 strains), *A. parasiticus* (2), *F. oxysporum* (2) and *F. verticillioides* (3) possessed the moderate lipase production. On the other hand, the low ability of lipase activity was recorded in 11 strains of *A. flavus*, 2 of *F. oxysporum* and one of each *A. parasiticus*, *F. verticillioides* and *A. nidulans*. Twenty-five out of 52 yeast strains represented 48.07 % of total yeasts showed positive results where 18 and 7 possessed moderate and low activities of lipases respectively. The moderate producers were belonging to *Candida* (5 strains), *Pichia* (4), *Trichosporon* (3), *Magnisiomyces* (2) *Cryptococcus*, *Galactomyces, Lethecophora* and *Yarrowia* (1 strain each). The low producers of lipase were 3 strains of *Trichosporon* and one of each of *Candida*, *Cryptococcus*, *Kluyveromyces* and *Pichia*. (Table 1).

In agreement with the present findings, **Mohawed *et al.* (1985)** found that 24 strains of *Aspergillus* had lipolytic activity and the most active was *A. niger*. **Banwart (1989)** reported that species of *Aspergillus, Penicillium, Fusarium, Cladosporium, Geotrichum, Mucor, Alternaria* and *Rhizopus* were lipase producers. **Magan *et al.* (1993)** stated that species of *Aspergillus*, *Eurotium*, and *Penicillium* could produce lipases at both 15 °C and 25 °C on tributyrin agar. **Savitha *et al.* (2011)** found only 4 (three of *Aspergillus* and one of *Mucor*) out of 32 isolates were positive for lipase production.

Working with cow milk and cheese, **Chipilev *et al*. (2016)** isolated 122 mold strains and reported that the strongest lipolysis was exhibited by the representatives of the *Geotrichum*, *Mucor* as well as a few species of the *Aspergillus* genus. Strong lipolytic activity was exhibited by 17 strains of *Geotrichum***,** They also observed that 43 (74.1%) of the molds in raw milk and 47 (73.4%) of the molds in cheese were distinguished by strong lipolytic activity. Also, Lipolysis of long chain fatty acids has been demonstrated for *Y. lipolytica, C. catenulata,* and *C. geotrichum* and the activity had the same level for these yeasts **(Roostita and Fleet 1996; van den Tempel 2000).**

b) Proteolytic activity

Forty-one strains comprising 82 % of total filamentous fungi tested showed high abilities of protease production (Table 1). *Aspergillus flavus* has been the most potent in protease production since 15 out of 20 tested strains, were high protease producers while the remaining 5 could produce the enzyme in a moderate level. *A. niger* was the runner up of *A. flavus* in the protease production and 80 % of its strains (8) could produce high level of the enzyme. All strains of *A. nidulans*, *F. oxysporum* and *F. verticillioides* in addition to 2 strains of *A. parasiticus* were high protease producers. The remaining 9 strains of filamentous fungi were of moderate proteolytic activity (Table 1).

With respect to yeast strains 76.47 % of total yeasts tested showed low to high abilities of protease production. *Candida parargosa*, *C. zeylanoides*, *Pichia cactophila*, *P. kudriavzevii*, *Cryptococcus curvatus*, *Galactomyces candidum* and *Lethecophora* sp. were active producers of protease (Table 1). Sixteen strains belonging to 15 species could secrete the protease enzyme in moderate amounts, these were *Candida tropicalis* (2 strains), and *C. parapsilosis, Clavispora lusitaniae, Cryptococcus curvatus, C.liquifacence, Galactomyces candidum, Kodamaco ohamire, Lecythophora sp., Magnsiomyces capitatus, Pichia cactophila, P. mansurica, Trichosporon asahii, T. insectorum, T. jirovecii* and *Yarowiiai lipolotica* (one strain each). Sixteen strains were low producers while the remaining 12 strains of yeasts could not produce detectable amounts of protease enzyme (Table 1).

Production of proteases by fungi belonging to the genera *Aspergillus* (**Paranthaman *et al.* 2009; Vishwanatha *et al.* 2010 a and b**) and *F. verticillioides* (**Facchini *et al.* 2015**) was reported. The proteolytic activity of *Penicillium*, *Mucor*, *Cladosporium* and *Aspergillus* was observed by **Abdel-Rahman and Saad** (**1989**) with *Mucor* and *Cladosporium* spp. showing the highest activity. **Chandrasekaran *et al.* (2015)** reported that *Aspergillus flavus* and *Aspergillus niger* showed highest protease activity during a screening of fungi for the ability to produce protease enzyme. **Grieve *et al.* (1983)** showed that a strain of *K. lactis* was able to hydrolyze casein importance maturation of cheese.

Gueguen and Lenoir (1975) examined 30 strains of *G. geotrichum* and found that they produced extracellular and intracellular proteinases and peptidases. Extracellular activity was present in 66% of the strains, which were divided into two groups of 25% showing high proteolytic activity and 75% having low proteolytic activity.

Mlimbila *et al.* (2013) tested 25 yeast strains from yoghurt for protease production and found that 21(84%) were protease positive. The highest proteolytic strains were belonging to the genus *Candida* of which *C. pararugosa* and *C. tropicalis* were the strongest protease producers.

A lot of researchers tested both lipolytic and proteolytic activities for filamentous and yeast fungal strains. **Nasser (2002) and El-Diasty (2004)** found that *Aspergillus* spp., *Penicillium* spp. and *Candida* spp. possessed proteolytic and lipolytic activities. **El-Diasty and Salem (2007)** reported that *A. flavus*, *A. niger*, *Mucor* spp. and *Penicillium* spp. exhibited proteolytic activities with different strength, and lipolytic activities for *Geotrichum* spp., *Yarrowia lipolotica* and *Candida parapsilosis*. The lipolytic and proteolytic activities of fifty-eight fungal isolates recovered from camel milk, were tested by **Korashy and Wahbba (2008**) who reported that isolates belonging to *Aspergillus, Absidia, Rhizopus, Fusarium* and yeasts secreted lipase and protease enzymes, and the most active producers *A. niger* and *A. flavus*. According to **Pitt and Hocking (2009)** *C. parapsilosis* is considered a dangerous spoilage organism in dairy products due to the ability to produce lipolytic and proteolytic enzymes. **Cardoso *et al.* (2015)** reported that among the predominant yeast strains contaminating Brazilian cheese, *Kodamaea* *ohmeri* and *Kluyveromyces marxianus* showed low lipolytic and high proteolytic activity.

Nevertheless, proteolysis and lipolysis of milk and milk food products containing could have a significant impact on their quality, promoting a rancid flavour and alterations in product texture. *Yarrowia lipolytica* has the ability and produce lipolytic and proteolytic enzymes **(Vasileva-Tonkova *et al*. 1996)**, which enables it to hydrolyze milk fat and protein and gives an unwanted texture of cheese due to degradation of fat. (**Corbaci *et al* 2012, Westall and Filtenborg, 2010**).

2-Mycotoxin production by filamentous fungi

In the present study 17 out of 20 strains (85%) of *A. flavus* isolated from raw cow milk, raw buffalo milk, yoghurt, Kareich cheese and soft cheese produced variable levels of aflatoxins B_1 and G_1. Eight of them were of moderate capability, giving 50 - 100 µg/l and 9 were of low activity yielding less than 50 µg/l (Table 1). All four isolates of *A. parasiticus*, recovered from raw cow and buffalo milk, could produce aflatoxins B_1 and G_1 in moderate (50-100 µg/l) levels. Isolates of *A. niger* were not able to produce any detectable amount of mycotoxins. In agreement with the current results, **Chipilev *et al*. (2016)** tested 11 strains of *A. flavus* obtained from milk and white cheese and found that only 2 isolates (18.1 %) had the ability to produce aflatoxin B_1. Also, **El Bagoury and Mosaad (2002)** reported that strains of *A. flavus* were AFB_1 producers. **Hoeltz *et al* (2005)** tested 30 isolates of *A. flavus* isolated from cheese and found that only 12 isolates (40 %) were positive for aflatoxin B_1 production. Also, **Ritter *et al*. (2011)** examined 30 isolates of *A. flavus* from cheese and rice samples and he found that only 8 isolates (27 %) produce aflatoxin B_1. On the other hand, **Abarca *et al*. (1994)** reported that strains of *A. niger* have the ability to secretly ochratoxin A in milk.

In the present investigation five out of six strains of *A. nidulans* were able to produce different levels of sterigmatocystin. Strain No. AUMC 11499 recovered from soft cheese could produce sterigmatocystin in a high level, Another strain (AUMC 11500) from yoghurt produced moderate (50-100 µg/l) level. Lower levels of sterigmatocystin were detected in culture extracts from strains AUMC 11497, AUMC 11498 and AUMC 11501 isolated from cow milk (Table 1).

No reports are available on the production of sterigmatocytin by *A. nidulans,* recovered from milk and milk products. **Abdel-Kareem (2010)** extracted sterigmatocystin from *A. nidulans* recovered from Egyptian fruit Juices. **Lund *et al*. (1995)** isolated sterigmatocystin from cheese samples contaminated with *A. versicolor*. In the current work, *F. oxysporum* (4 strains) and *F. verticillioides* (6 strains), were examined for their ability of mycotoxin production. Two strains of *F. verticillioides* (AUMC 11505 and AUMC 11506) isolated from yoghurt could produce fumonisin at lower (less than 100 µg/l) level, and two isolates of *F. oxysporum* (AUMC 11508 and AUMC 11511) recovered from buffalo and cow milk, respectively, were zearalenone producers at low levels. **Refaie (2013)** found that two out of four strains of *F. verticillioides* from processed meat produced low levels of fumonisins. Also, she examined five strains of *F. oxysporum* for Zearalenone production and found that only one produced Zearalenone with low level.

Fumonisins are possibly carcinogenic to humans, and according to the International Agency for Research on Cancer, they rated as class 2 B carcinogens (**WHO, IARC 1993**).

Table 1: Lipolytic, proteolytic and toxinogenic activities of molds isolated from milk and milk products.

Fungal species	AUMC	Source	Lipase activity		Protease activity		Mycotoxins	
			Depth (mm)	Levels	Depth (mm)	Levels	Type	Levels
Aspergillus flavus	11462	Cow milk	3	L	22	M	-	N
	11463	Cow milk	-	-	18	M	B_1 and G_1	M
	11464	Cow milk	5	L	27	H	B_1 and G_1	M
	11465	Buffalo milk	2	L	20	M	B_1 and G_1	M
	11466	Buffalo milk	5	L	32	H	B_1 and G_1	L
	11467	Buffalo milk	2	L	33	H	B_1 and G_1	L
	11468	Yoghurt	6	L	27	H	-	N
	11469	Yoghurt	7	M	28	H	B_1 and G_1	L
	11470	Yoghurt	8	M	26	H	B_1 and G_1	L
	11471	Kareish	11	M	16	M	B_1 and G_1	L
	11472	Kareish	3	L	27	H	B_1 and G_1	L
	11473	Kareish	8	M	27	H	B_1 and G_1	L
	11474	Soft cheese	6	L	26	H	-	N
	11475	Soft cheese	7	M	30	H	B_1 and G_1	L
	11476	Soft cheese	4	L	31	H	B_1 and G_1	L
	11477	Cow milk	4	L	30	H	B_1 and G_1	M
	11478	Buffalo milk	2	L	28	H	B_1 and G_1	M
	11479	Yoghurt	13	M	22	M	B_1 and G_1	M
	11480	Kareish	7	M	33	H	B_1 and G_1	M
	11481	Soft cheese	7	M	26	H	B_1 and G_1	M
A. parasiticus								
	11482	Cow milk	5	M	22	M	B_1 and G_1	M
	11483	Cow milk	-	-	31	H	B_1 and G_1	M
	11484	Buffalo milk	6	L	27	H	B_1 and G_1	M

Fungal species	AUMC	Source	Lipase activity		Protease activity		Mycotoxins	
			Depth (mm)	Levels	Depth (mm)	Levels	Type	Levels
	11485	Buffalo milk	7	M	19	M	B_1 and G_1	M
A. niger	11486	Cow milk	26	H	27	H	-	N
	11487	Cow milk	23	H	32	H	-	N
	11488	Buffalo milk	22	H	15	M	-	N
	11489	Buffalo milk	28	H	30	H	-	N
	11490	Yoghurt	27	H	35	H	-	N
	11491	Yoghurt	26	H	32	H	-	N
	11492	Kareish	26	H	30	H	-	N
	11493	Kareish	28	H	20	M	-	N
	11494	Soft cheese	20	H	33	H	-	N
	11495	Soft cheese	26	H	35	H	-	N
A. nidulans	11496	Cow milk	-	-	44	H	_	N
	11497	Cow milk	-	-	45	H	Sterigmatocystin	L
	11498	Cow milk	-	-	42	H	Sterigmatocystin	L
	11499	Soft cheese	-	-	38	H	Sterigmatocystin	H
	11500	Yoghurt	2	L	46	H	Sterigmatocystin	M
	11501	Kareish	-	-	47	H	Sterigmatocystin	L
Fusarium verticillioides	11502	Cow milk					_	N
	11503	Cow milk	10	M	30	H	_	N
	11504	Buffalo milk	3	L	43	H	_-	N
	11505	Yoghurt	12	M	40	H	Fumonisin	L
	11506	Yoghurt	-	-	38	H	Fumonisin	L
	11507	Kareish	10	M	34	H	_-	N
F. oxysporum	11508	Buffalo milk	-	-	40	H	Zearalenone	L
	11509	Buffalo milk	10	M	33	H	_	N
	11510	Cow	6	L	33	H	_	N

Fungal species	**AUMC**	**Source**	**Lipase activity**		**Protease activity**		**Mycotoxins**	
			Depth (mm)	**Levels**	**Depth (mm)**	**Levels**	**Type**	**Levels**
		milk						
	11511	Cow milk	5	L	43	H	Zearalenone	L

Mycotoxin: **H**= High level (more than 100 µg/l medium), **M**=Moderate level (50 - 100 µg/l medium) **L** = Low level (less than 50 µg/l medium and **N**= Non-producer
Protease and lipase: **H**= High more than 20 mm, **M**= Moderate 10-20 mm, and **L**=Low less than 10 mm
NT: Not tested

Table 2: Lipolytic and proteolytic activities of yeasts isolated from milk and milk products.

Fungal species	**AUMC**	**Source**	**Lipase activity**		**Protease activity**	
			Depth (mm)	**Levels**	**Depth (mm)**	**Levels**
Candida catenulata	11069	Buffalo milk	-	-	-	-
C. parapsilosis	11090	Soft cheese	-	-	22	M
C. parargosa	11073	Buffalo milk	9	M	30	H
C. pseudopararogosa	11062	Buffalo milk	-	-	10	L
C. tropicalis	11055	Cow milk	5	L	14	M
	11080	Kareish	11	M	18	M
	11101	Yoghurt	7	M	-	-
C. zeylanoides	11054	Buffalo milk	8	M	26	H
	11074	Buffalo milk	11	M	6	L
Clavispora lusitaniae	11078	Yoghurt	-	-	13	M
	11079	Soft cheese	-	-	-	-
	11106	Kareish	-	-	4	L
C. curvatus	11114	Buffalo milk		10	M	40
	11051	Buffalo milk		-	-	15
C.liquifacence	11064	Cow milk		6	L	13
Glactomyces candidum	11070	Cow milk	-	-	3	L
	11085	Kareish	-	-	14	M
Geotrichum candidum	11089	Kareish	12	M	27	H
Kluyveromyces lactis	11095	Kareish	-	-	10	L
	11099	Kareish	6	L	7	L

Fungal species	**AUMC**	**Source**	**Lipases activity**		**Proteases activity**	
			Depth	**Levels**	**Depth**	**Levels**

			(mm)		(mm)	
Kluyveromyces marxianus	11097	Yoghurt	-	-	-	-
Kodamaco ohamire	11053	Buffalo milk	-	-	18	M
Lecythophora sp.	11098	Kareish	8	M	38	H
	11059	Buffalo milk	-	-	18	M
Magnsiomyces capitatus	11077	Cow milk	7	M	20	M
Magnsiomyces sp.	11081	Yoghurt	11	M	-	-
Pichia anómala	11093	Kareish	-	-	10	L
P. cactophila	11083	Soft cheese	12	M	13	M
	11067	Cow milk	8	M	29	H
P. mansurica	11104	Soft cheese	-	-	12	M
P. membranifacenss	11096	Soft cheese	-	-	10	L
	11107	Soft cheese	12	M	-	-
Rhodotorula mucilaginosa	11117	Soft cheese	-	-	-	-
	11057	Cow milk	-	-	-	-
T. asahii	11091	Kareish	6	L	10	L
	11052	Buffalo milk	-	-	11	L
	11047	Cow milk	-	-	8	L
	11105	Yoghurt	-	-	13	M
T. insectorum	11048	Cow milk	6	L	-	-
	11092	Soft cheese	12	M	14	M
T. jirovecii	11061	Cow milk	8	M	16	M
	11115	Cow milk	-	-	6	L
T. loubieri	11068	Cow milk	10	M	5	L
	11072	Buffalo milk	6	L	7	L
Yarowiiai lipolotica	11111	Soft cheese	-	-	8	L
	11116	Soft cheese	9	M	15	M

Protease and lipase: **H**= High more than 20 mm, **M**= Moderate 10-20 mm, and **L**=Low less than 10 mm **NT:** Not tested

REFERENCES

Abarca M. L., Bragulat M. R., Castella G. and Cesahan F. J. (1994): Ochratoxin A production by strains of *Aspergillus niger* var. *niger*, Applied and Environmental Microbiology. 60: 2650 – 2652.

Abdel-Kareem M. M. (2010): Microbiota and mycotoxins occurrence in some Egyptian fruit Juices. M. Sc. Thesis, Botany Department, Faculty of Science, Sohag University, Sohag, Egypt.

Abdel-Rahman A. H. and Saad S. M. (1989): Studies on the proteolytic activity of some prevalent mould species in relation to meat hygiene. Zagazig Veterinary Medical Journal. 17: 228-236.

Banwart G. J. (1989): Basic Food Microbiology 2nd Ed. An Avi book published by Van Nostrand Rein hold New York. 781 pages.

Cardoso V. M. , Borelli B., Lara C. A. and Rosa C. A. (2015): The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese. Food Research International 69 :331-340.

Chandrasekaran S., Kumaresan S. S. P., Manavalan M. (2015): Production and Optimization of Protease by Filamentous Fungus Isolated from Paddy Soil in Thiruvarur District Tamilnadu. Journal of Applied Biology and Biotechnology 3:66-69.

Chipilev N., Daskalov H. and Stoyanchev T. (2016): Study on the prevalence of lipolytic yeasts and moulds in raw cow milk and white brined cheese. Bulgarian Journal of Veterinary Medicine, 19 (2): 117–126.

Conesa A., Punt J., Luijk N. and Hondel C. A. (2001): The secretion pathway in filamentous fungi; a biotechnological view. Fungal Genetics and Biology 33 (3): 155-171.

Corbaci C. F., B. Ucar and Yalcin H. T. (2012): Isolation and characterization of yeasts associated with Turkish-style homemade dairy products and their potential as starter cultures. African Journal of. Microbiology Research. 6: 534-542.

Cormie S. (1992): Psychrotrophs and their enzyme residues in cheese milk. A review. Australian Journal of Dairy Technology. 47:96-100.

Downey W. K. (1980): Flavour impairment from pre- and post manufacture lipolysis in milk and dairy products. A review. Journal of Dairy Research 47: 237-252.

El-Bagoury A. M. and Mosaad A.A. (2002): Mycological quality of yoghurt with special reference to aflatoxigenic mould. Minufyia Veterinary Journal. 2 (1):69-77.

El-Diasty E. M. (2004): Study on mycological quality of fish. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ., Beni-Suef Branch.

El-Diasty E. M. and Salem R. M. (2007): Incidence of Lipolytic and Proteolytic Fungi in Some Milk Products and Their Public Health Significance. Journal of Applied Sciences Research, 3(12): 1684-1688.

EL-Kady I. A. and Moubasher M. H. (1982): Toxigenicity and toxin of Stachybotrys chartarum isolates from wheat straw samples in Egypt. Experimental Mycology. 6: 25-31.

Facchini FDA, Vici AC, Pereira MG, Jorge JA, Maria de Lourdes T.M. and Polize I: (2015): Enhanced lipase production of *Fusarium verticillioides* by using response methodology and wastewater pretreatment application. Journal of Biochemical Technology. 6(3): 996-1002.

Frisvad J. C., Skouboe P. and Samson R. A. (2005): Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B1, sterigmatocystin and 3-O-methylsterigmatocystin, *Aspergillus rambellii sp*. nov. Systematic Applied Microbiology. 28: 442–453.

Gourama N. and Bullerman L. B. (1995): *Aspergillus flavus* and *Aspergillus parasiticus*: Aflatoxigenic fungi of concern in foods and feeds: A review. Journal of Food Protection. 58: 1395–1404.

Grieve P. A., Kitchen B. J., Dulley J. R. and Bartley J. (1983): Partialn characterization of cheese-ripening proteinases produced by the yeast Klu*yveromyces lactis*. Journal of Dairy Research. 50: 469–480.

Gueguen M. and Lenoir J. (1975): Aptitude de l'espe`ce Geotrichum candi-dum a` la production d'enzymes prote´olytiques. Lait 55:145–162.

Hoeltz, M. Estudo da Influência de Manejos Pós-Clolheita na Incidência de Fungos e Micotoxinas no Arroz (Oryza sativa L.). (2005): 77 f. Dissertação (Mestrado em Microbiologia Agrícola e do Ambiente)-Universidade, Federal do Rio Grande do Sul, Porto Alegre, 1: 77-79

Karanam S. K., Medicherla N. R. (2008): Enhanced lipase production by mutation induced *Aspergillus japonicus*. African Journal of Biotechnology. 7(12):2064-2067.

Kashmiri M. A., Ahmad A. and Butt B. W. (2006): Production, purification and partial characterization of lipase from *Trichoderma viride*. African Journal of. Biotechnology. 5(10):878-882.

Khalifa M. I., Al-Ashmawy M. A., Abdel-Khalik A. and El-Sherbini M. (2013): Mycological evaluation of serving some dairy products with specific reference to mycotoxins production in Azhar University Student Hostels.World Journal of Dairy and Food Sciences 8:165-170.

Korashy E. and Wahbba M. N. (2008): Lipolotic and Proteolytic Activities of some fungi isolated from raw camel`s milk. Assiut Veterinary Medical Journal. 54 (118): 135-146

Lima V. M. G., Krieger N., Sarquis M. I. M., Mitchell D. A., Ramos L. P. and Fontana J. D (2003): Effect of nitrogen and carbon sources on lipase production by Penicillium aurantiogriseum. Food Technology and Biotechnology. 41(2):105- 110.

Lund F., Filtenborg O. and Frisvad J. C. (1995): Associated mycoflora in cheese. Food microbial. 12:173-180.

Magan N., Nina E. and Howarth J. J. (1993): Lipolytic activity and degradation of rapeseed oil and rapeseed by spoilage fungi. International journal of food microbiology. 19(3):217-227.

Mlimbila J. **S., Hosea K. M., and Muruke M. (2013):** Molecular Identification and Proteinase Activity of Yeasts Isolated From Fermented Milk International Journal of Diary Science Research, 2 (2): 7-14.

Mohawed S.M., Abdel-Fattah N.K. and El-Shahed A.S. (1985): Studies on the lipolytic activity of some local isolates of Aspergilli. Proc. Egypt. Bot. Soc. 4 (lsmaillia Conf.). pp. 68-80.

Nasser L. A. (2002): “Mycological status of imported canned fish consumed in Saudia Arabia with special reference to proteolytic activity”. Assiut Veterinary Medical Journal., 47: 125-130.

Paranthaman R, Alagusundaram K and Indhumathi J (2009): Production of Protease from Rice Mill Wastes by *Aspergillus niger* in Solid State Fermentation. World Journal of Agricultural Sciences 5 (3): 308-312.

Paterson R. R. M. and Bridge P. D. (1994): Biochemical techniques for filamentous fungi. Int. Mycol. Instit. CAB International Survey. UK. 125 pages.

Pitt J. I. and Hocking A. D. (2009): Fungi and Food spoilage (3rd Ed.). Springer Science Business Media, LLC, 233 Spring Street, New York, NY 10013, USA.

Refaie (2013): Studies on fungi deteriorating processed meat products. M.Sc., Department of Botany and Microbiology, Faculty of Science, Assiut University, Egypt.

Ritter A. C., Hoeltz, M. and Noll B. (2011): Toxigenic potential of *Aspergillus flavus* tested in different culture conditions. Ciênc. Tecnol. Aliment. (Ciência e Tecnologia de Alimentos), Campinas, 31(3): 623-628.

Roostita R. and Fleet G. H., (1996): The occurrence and growth of yeasts in Camembert and Blue-veined cheeses. International Journal of Food Microbiology. 28: 393–404.

Savitha S, Sadhasivam S, Swaminathan K and Lin F. H. (2011): Fungal protease: Production, purification and compatibility with laundry detergents and their wash performance. Journal of the Taiwan Institute of Chemical Engineers. 42:298–304.

Sengum I., Yaman D. and Gonul S. (2008): Mycotoxins and mould contamination in cheese.World Mycotoxicology Journal 3:291-298.

Sweeney M. J. and Dobson A. D. W. (1998): Mycotoxin production by *Aspergillus, Fusarium* and *Penicillium* species. International. Journal of Food Microbiology 43: 141-158.

Ullman U. and Blasins C. (1974): A simple medium for the detection of different lipolytic activity of microorganisms. Zbl. Bakt. Hyg. II Abt Orig. A., 229: 264-267.

Van den Tempel, T. (2000):. The Role of Yeast in the Ripening of Blue Mould Cheese. (Thesis) The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Vasileva-Tonkova E., Balasheva D. M. and Galabova D. (1996): Influence of growth temperature on the acid phosphatase activity in the yeast *Yarrowia lipolytica*. FEMS Microbiol Lett. 145: 267–271.

Vishwanatha K. S., Appu Rao A. G. and Singh S. A. (2010a): Production and characterization of a milk-clotting enzyme from *Aspergillus oryzae* MTCC 5341. Appl Microbiol Biotechnol 85:1849-1859.

Vishwanatha K. S., Rao A. G., Singh S. A. (2010b): Acid protease production by solid-state fermentation using *Aspergillus oryzae* MTCC 5341: optimization of process parameters. Journal of Industrial Microbiology and Biotechnology 37:129-138.

Westall S. and Filtenborg O. (2010). Yeast occurrence in Danish feta cheese. Food Microbiol., 15 (1): 215–222.

WHO-IARC (1993): Toxins derived from *Fusarium moniliforme*: Fumonisin B_1 and B_2 and fusarin C. Lyon (F): IARC. World Journal of Agricultural Sciences.

Wickerham L. J. (1951): Taxonomy of yeasts. Tech. Bull.Nr.1029 Dep. Agr., Washington D. C.

Zohri A. A., Moharram A. M. and Refaie R. R. S. (2014): Mycobiota contaminating beef burger and sausage with reference to their toxins and enzymes. Journal of Basic and Applied Mycology. 5:61-73.

الأنزيمات والسموم المنتجة بواسطة الفطريات المعزولة من الألبان ومنتجاتها فى أسيوط، مصر

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تعتبر الألبان ومنتجاتها مصدراً من مصادر الحياه لإحتوئها على جميع العناصر الغذائية الهامه لجسم الأنسان إضافه الى كثير من المعادن والفيتامينات .لذلك ذاد فى الأونة الأخيره الإهتمام بجوده اللبن الخام ومنتجاته وخلو هذه المنتجات من بعض الملوثات الفطرية. لذا اهتمت هذه الدراسه بإختبار قدره بعض العزلات الفطرية (١٠٢ عزله:٥٠ عزله للفطريات الخيطيه و٥٢ عزله من الخمائر) معزوله من اللبنى البقرى الخام واللبن الجاموسى الخام وكذلك الزبادى و الجبنه القريش والجبنه البيضاء على إفراز أنزيمى الليبيز والبروتيز المحللين للدهون و البروتينات متسببه فى إفساد اللبن الخام ومنتجاته من حيث الطعم والرائحه وتقصير فتره صلاحيه المنتجات.

وقد اوضحت النتائج ان اكثر العزلات إنتاجيه لكلا الليبيز والبروتيز تنتمى الى كانديدا باراجوزا،كانديدا زيلانويد،كريبتوكوكس كرفاتس، فيوزاريم اوكسسبورم (من اللبن الجاموسى)،ماجنزيومايسيس كابيتاتس،تريكوسبورون جيروفيسى (من اللبن البقرى)،كانديدا تروبيكالس، جيوتريكم كانديدم ، ليسيثوفورا سبيشيس، بيكيا

كوادريافيزيفياى، اسبيرجيلس فلافس (من الجبن القاريش)، بيكيا كاكتوفيلا، تريكوسبورن إنسيكتورم و ياروبا ليبولوتيكا (من الجبن البيضاء)، اسبيرجيلس نيجر و فيوزاريم فيرتيسيلويدس (من الزبادى). كما القت هذه الدراسه الضوء على قدره العزلات الفطريه الخيطيه المعزوله من البن الخام ومنتجاتها على إنتاج السموم الفطريه التى تعد من أهم وأخطر الأضرار إذاتم إنتاج هذه السموم فى الألبان ومنتجاتها لما تسببه هذه السموم من اضرار وامراض مزمنه للإنسان وخسائر اقتصاديه لهذه المنتجات.

لذلك تم إجراء الإختبار على عدد ٥٠ عزله من عزلات الفطريات الخيطيه المعزوله مسبقا من اللبن الخام ومنتجاته، وكانت النتائج كالتالى:

تم إنتاج السموم الفطريه من حوالى ٣٠ عزله (٦٠%) من إجمالى العزلات التى تم إختبارها حيث تم إنتاج الأفلاتوكسين B1 و G1 من الأسبيرجيلس فلافس (١٧ عزله) ومن اسبيرجيلس برازيتيكس (٤ عزلات) معزولين من البن الخام وبعض منتجات الألبان. ايضا تم إنتاج الإستريجماتوسيستين من خمس عزلات من اسبيرجيلس نيديولانس وكذلك تم إنتاج الفيومونسين و الزيارالينون من بعض عزلات الفيوزاريوم اوكسيسبورم وفيوزاريوم فيرتيسيلويدس على التوالى.