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Comparative Mycological Assay on Prevalence of Yeasts, Molds and Aflatoxin M_1 (AFM_1) in Some Fermented Milk Products in Alexandria, Egypt

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Abstract: Fermented milk; rayeb and yoghurt; harbored a high risk of spoilage by yeasts and molds; as they were able to grow in a broad range of pH environments; as well as by aflatoxin M1 (AFM_1) which is a hepatic carcinogenic metabolite, those fungi introduced into these milk products as contamination during the milking process followed by no heat treatment or as recontamination after the heat treatment leading not only to product spoilage but also to very serious illnesses and food poisoning. A total of 120 random samples of locally manufactured fermented milks including; 40 samples of rayeb milk (plain and sterilized; 20 each), 40 samples of plain yoghurt and 40 samples of stirred yoghurt (20 strawberry and 20 peach yoghurt) were purchased from vendors in their retail containers as sold to the public in Alexandria governorate, Egypt, during the period extended from December, 2015 till February, 2016. Samples subjected to mycological analysis including counting, isolation and identification of yeasts and molds beside determination of Aflatoxin M_1 (AFM_1) mycotoxin in a total number of 15 rayeb and yoghurt samples by Enzyme Linked Immunosorbant Assay (ELISA) for the detection of AFM_1, that were divided into 3 samples from each of plain rayeb milk and yoghurt, sterilized rayeb milk, strawberry and peach yoghurt. It was recorded that the mean value of yeasts count were $8.12\times10^5 \pm 3.10\times10^2$ and $7.04\times 10^5 \pm 2.13\times10^2$ CFU/ml for plain and sterilized rayeb, respectively. On the other hand, the mean value of molds count were $4.45 \times 10^5 \pm 2.14 \times 10^2$ and $3.53 \times 10^5 \pm 2.45 \times 10^2$ CFU/ml for plain and sterilized rayeb, respectively. Statistical analysis of the obtained results clarified that there was a significant difference ($P\leq0.05$) in mean values of yeasts and molds counts of the examined samples of plain and sterilized rayeb and means values of yeast count was higher than that mean values of molds count. Also, it was found that the mean values of yeasts count in the examined samples of plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt were $8.37 \times 10^5 \pm 2.4 \times 10^2$, $7.23 \times 10^5 \pm 2.55 \times10^2$ and $7.40 \times 10^5 \pm 3.52 \times 10^2$ CFU/ml, respectively. On the other hand, the mean values of molds count in the examined samples of plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt were $3.09 \times 10^5 \pm 1.5 \times 10^2$, $3.83 \times 10^5 \pm 1.77 \times 10^2$ and $3.42 \times 10^5 \pm 3.41 \times 10^2$ CFU/ml. Mycological identification revealed the isolation of serious health threatspathogens from examined fermented milk products as *Aspergillu sflavus, Aspergillus parasiticus, Aspergillusnige*, *Candida albicans* and others. In addition, Aflatoxin M_1 was determined in plain and sterilized rayeb at the rate of 11.0 ± 1.39 and 0.020 ±0.003 mg/kg, respectively, while itwas determined in plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt at the rate of 20.0 ± 3.69, 0.042 ± 0.006 and 0.007 ± 0.0001 mg/kg, respectively with a significant difference ($P\leq0.05$) among different examined samples that highlighted the possible adverse cumulative effect of AFM1 by consuming suchcontaminated products. According to the Egyptian Standards, it was noticed that all of the examined samples of rayeb and yoghurt in the current study failed to comply with the Egyptian Standards that constitutes a high risk health hazard to consumers so safety measures should be considered to avoid potential threats and educational programs should be applied for producers as well as HACCP system (Hazard analysis and critical control point) to prevent or at least reduce contamination by these pathogens and to apply strict hygienic measures during production, storage and distribution of the fermented milk products.

Key words: *Yeasts – Molds – AFM1 – ELIZA – Rayeb- Yoghurt.*

1. Introduction

Milk and products derived from milk of dairy cows can harbor a variety of microorganisms and can be important sources of foodborne pathogens. There are many causes for presence of zoonotic foodborne pathogens in milk such as direct contact with contaminated sources in the dairy farm environment and to excretion from the udder of an infected animal. Furthermore, some pathogens can survive and thrive in post-pasteurization processing environments, thus leading to recontamination of dairy products. These pathways pose a risk to the consumer from direct exposure to foodborne pathogens present in unpasteurized dairy products as well as dairy products that become re-contaminated after pasteurization **(Oliver *et al.,* 2005).** The consumption of fermented milks by man is dated from the beginning of civilization, once residues of these products were found in pottery fragments from Neolithic Bronze and iron ages settlements in Britain **(Mckinley, 2005).** Yoghurt is a popular fermented milk product consumed in many parts of the world. It is an extremely popular fermented milk food in Europe, Asia and Africa **(Thapa, 2000).** Fermented milk products were dairy foods that have been fermented with lactic acid bacteria such as *Lactobacillus*, *Lactococcus*, and *Leuconostoc*. The fermentation process increased the shelf-life of the product, while enhancing the taste and improving the digestibility of milk. Various types of fermented milks and derived products have been developed in all parts of the world, each with its own characteristic history. Their nature depended on type of the used milk, the pre-treatment of milk, climate and the conditions of fermentation **(Tamine and Robinson, 2007).** Moreover,fermented milks were very versatile products that suit all plates and meal occasions.

Frazier and Westhoff (2001) report that *Candida* species have been used with dairy starter cultures in rayeb to maintain the activity and increase the longevity of LAB. This could imply a symbiotic association between *Candida* species and the LAB involved in Leben production. Indeed, a symbiosis between yeasts and lactic acid bacteria has been suggested: whereby the bacteria provide the acidic condition favourable for the growth of yeasts. Rayeb milk is a traditional fermented milk product popular in rural areas of Egypt; Rayeb milk is traditionally made from raw buffalo milk by spontaneously fermentation. The raw milk is left to sour spontaneously at room temperature until it coagulate; it contains a mixed culture of lactic acid bacteria and other fermentative organisms **(Abd El Gawad *et al.* (2010).**

Yoghurt is low in saturated fat and cholesterol but nutritionally rich in protein and vitamins including pantothenic acid and riboflavin. Also, it is a very good source of calcium, iron, potassiumand phosphorus that maintains the red blood cells, prevents high blood pressure and helps keeping nervous system functioning well. In addition, yoghurt has antimicrobial activity to some bacteria **(Schulz and Hingst, 2000).** Usually fruit flavour and colour is added into yoghurt after fermentation. Contamination by yeasts is generally related to the fruits added and/or poor hygienic practices during packaging operation **(Fleet, 1990).** Yogurts are available in a vast array of flavors including fruit (apple, apricot, black cherry, black currant, blue berry, lemon, mandarin, raspberry, strawberry, peach), cereal, vegetables, chocolate, vanilla, caramel, ginger, etc **(Direct, 2013).**

Fermented milk products are liable to contamination with different types of microorganisms from different sources during production, processing and handling, which lead them to be unfit for consumption and constitute a public health hazard **(Todaro *et al.,* 2013).** Poor hygiene, practiced by handlers of fermented milk products in local markets, may lead to spoilage with pathogenic microorganisms including fungi since they do not undergo further processing before consumption **(Tamine and Robinson, 2007).** The presence of yeasts and molds in dairy products is objectionable, as they resulted in spoilage of the product. Also, the presence of yeasts and mold in yoghurt reflects unsanitary hygienic measures in manufacturing and packaging thus in some countries, yeasts and mold counts are considered the standard test for checking factory sanitation **(Foster *et al.,* 1983).** Yeasts are a major cause of spoilage of yogurt and fermented milks in which the low pH provides a selective environment for their growth **(Rohm *et al.,* 1992).** Fungal spoilage of fermented milk products is manifested by presence of wide variety of metabolic by-products causing off-odors and flavors in addition to visible changes in color and texture. The most common spoilage species are *Aspergillus*, *penicillium*, *Rhizopus*, *Fusarium* and *Trichderma* that are known as spore formers **(Pitt and Hocking, 2009)**. The microbiological quality of fermented milks was investigated by many authors; **Ahmed (1995), Riad (1996), Shawer (1997), Adebesin *et al.* (2001), Abdel All and Dardir (2009), El-Malt *et al.* (2013), El-Ansary (2014), Dirisu *et al.* (2015), Ibrahim *et al.* (2015)** and **Samet-Bali *et al.* (2016)**.

Aflatoxins, as known, are produced mainly by the *Aspergillus* species; *A. flavus* and by most, if not all, strains of *A. parasiticus*, plus related species, *A. nomius* and *A. niger*. Also, investigations revealed that there are 4 major aflatoxins: B1, B2, G1, and G2 plus 2 additional metabolic products, M1 and M2, which are of significance as direct contaminants of foods and feeds. The AFM1 and AFM2 were first isolated from milk of lactating animals fed aflatoxin preparations; hence the M designation. Aflatoxin M1 (AFM1) is 4-hydroxy aflatoxin B1 and aflatoxinM2 is 4-dihydroxy aflatoxin B2 **(Doyle *et al.,* 1997).** Mycotoxins are secondary metabolites of molds that have adverse effects on humans, animals, and crops that result in illnesses and economic losses. Aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins, and ergot alkaloids are the mycotoxins of greatest agro-economic importance **(Bennett and Klich, 2003).** Nearly, 5 billion people in developing countries globally are at risk of chronic exposure to aflatoxinsvia contaminated feed **Shephard and Sewram (2004).** Aflatoxins are generally produced in animal feed by toxigenic fungi such as *Aspergillus flavus*, *Aspergillu sparasiticus* and *Aspergillu snomius* **(Chen *et al.,* 2005).** Aflatoxins have sub-acute and chronic effects such as liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis in humans, AFM_1 is classified in Group 2 as a probable human carcinogen **(Hampikyan *et al.,* 2010).** Many countries have regulations to control the levels of AFB_1 in feeds and to propose maximum permissible levels of AFM_1 in milk to reduce this risk **(Akkaya *et al.,* 2006).** The consumption of milk and dairy products by human especially by infants and young children increases the risk of exposure to AFM_1 **(Colak, 2007)**. AFM_1 remains stable after pasteurization, sterilization, preparation and storage of various dairy products (**Lin *et al.,* 2004**).

Aflatoxins, are the potent hepatotoxins and carcinogens known, their effects vary with duration of exposure and nutritional status. The clinical signs of acute aflatoxicosis are represented by acute hepatic necrosis, resulting later in cirrhosis or carcinoma of the liver. Acute hepatic failure is manifested by hemorrhage, edema, and alteration in digestion, changes to the absorption and/or metabolism of nutrients and mental changes and/or coma **(Barjesteh *et al.,* 2010),** sothe presence of aflatoxin M_1 in milk and dairy products was investigated by many authors; **Panahi *et al.* (2011); Aliabadi *et al.* (2012); Issazadeh *et al.* (2012)**; **Iha *et al.* (2013)** and **Kos *et al.* (2014).**

Therefore, the present study was designed to perform comparative mycological assay on prevalence of yeasts, molds and Aflatoxin M_1 (AFM_1) in some fermented milk products in Alexandria, Egypt including; plain rayeb, sterilized rayeb, plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt by counting, isolation and identification of yeasts and molds beside determination of Aflatoxin M_1 (AFM_1) mycotoxin by using ELISA.

2. Material and Methods

Collection of Samples:

A total of one hundred and twenty (120) random samples of locally manufactured fermented milks including; 40 samples of rayeb milk made by traditional method were collected aseptically (divided into, 20 purchased from vendors in duplicates as well as 20 stirred samples from trade companies), 40 samples of plain yoghurt and 40 samples of stirred yoghurt (strawberry and peach yoghurt; 20 of each). All samples were purchased from vendors in their retail containers as sold to the public in Alexandria governorate, Egypt during the period extended from December, 2015 till February, 2016. The data on samples labels including; date of manufacture, expire date, name of the product, types and ratio of the fruits additives were recorded. The samples were dispatched directly to the laboratory at 4-5°C by using of an icebox and stored in laboratory under refrigeration at 4°C until examination as soon as possible. Samples subjected to mycological analysis including counting, isolation and identification of yeasts and molds beside determination of Aflatoxin M_1 (AFM_1) mycotoxins in a total number of 15 rayeb and yoghurt samples by Enzyme Linked Immunosorbant Assay (ELISA) for the detection of AFM1, divided into 3 samples from each of plain rayeb milk and yoghurt, sterilized rayeb milk, strawberry and peach yoghurt.

Preparation of Samples:

It was performed according to **APHA, (2004)**

Preparation of examined Laban rayeb samples:

Samples of rayeb should be thoroughly mixed by a sterile stirrer. For mycological examination, 10 grams of the tested sample were weighted into a flask then 90 ml of sterile peptone water solution were added and mixed by sterile stirrer.

Preparation of yoghurt samples:

Samples of plain and sterilized flavored yoghurt in their containers ready for sale were thoroughly mixed by sterile stirrer. For mycological examination, eleven grams or ml of the tested sample were weighted into a flask then 99 ml of sterile peptone water solution were added and mixed by sterile stirrer. To obtain tenfold serial dilution 1/10, from which decimal dilutions up to 10^6 were prepared using sterile peptone water.

I) Mycological Examination:

1. Isolation and counting of yeasts and molds were performed according to **Baily and Scott (1998).**
2. Suspected yeasts-like colonies were identified according to the scheme given by **Terrence (1971).**
3. Purified cultures were identified biochemically using sugar fermentation, sugar assimilation and nitrate assimilation **(Larone, 1987)** as well as urease test **(Cruickshank *et al.,* 1973).**
4. The identification of isolated mold was carried out according to methods of **Larone (1987).**

5. The growth was identified macroscopically for colour, size, pigment, texture and reverses while microscopically for conidia, canidophore, phialids and head according to **Pitt and Hocking (1997)** for genus *Aspergillus*, and according to **Ramírez (1982)** for the genus *Penicillium*, while other genera were identified according to **Samson *et al.* (2007).**

II) Preparation of rayebsamples **(Kamkar *et al.,* 2011)** and yoghurt samples **(Baskaya *et al.,* 2006)** for determination of Aflatoxin M_1 (AFM_1) by ELISA according to **R-Biopharm (1999)**.

III) The obtained results were statistically analyzed by "ANOVA" that was conducting using SAS software

(SAS, 2014) P values of less than or equal to 0.05 were considered statistically significant.

3. Results

Results of yeasts and molds counts (CFU/ml) in the examined samples of rayeb milk:

Table (1): Statistical analytical results of yeasts and molds counts (CFU/ml)in samples of rayeb milk (n=20 of each).

Rayeb milk samples	CFU/ml	Positive samples		Minimum	Maximum	Mean ± SEM
		No.	%			
Plain rayeb	Yeasts	20	100	7.35×10^{3}	8.89×10^{3}	$8.12 \times 10^{3} \pm 3.10 \times 10^{2a}$
	Molds	20	100	4.11×10^{3}	4.78×10^{3}	$4.45 \times 10^{3} \pm 2.14 \times 10^{2c}$
Sterilized rayeb	Yeasts	20	100	6.93×10^{3}	7.14×10^{3}	$7.04 \times 10^{3} \pm 2.13 \times 10^{2b}$
	Molds	20	100	3.41×10^{3}	3.64×10^{3}	$3.53 \times 10^{3} \pm 2.45 \times 10^{2d}$

Values are three independent determination. Mean values having different superscript letters in a column for each sample are significantly different ($P \leq 0.05$).

Results of identified yeasts in the examined samples of rayeb milk:

Table (2):Frequency distribution of identified yeasts in examined samples of rayeb milk

Isolates of yeasts	Plain rayeb (n=20)		Sterilized rayeb (n=20)	
	No.	%	No.	%
Candida albicans	6	16.67	2	25.00
Candida krusei	5	13.89	1	12.50
Candida tropicalis	3	8.33	0	0.00
Candida stellotiodes	3	8.33	0	0.00
Candida guillormondi	1	2.78	0	0.00
Candida parapsillosis	2	5.56	1	12.50
Candida pseudotropicalis	1	2.78	1	12.50
Rhodoterullaglutinis	7	19.44	3	37.50
Rhodoterulla pichia	1	2.78	0	0.00
Rhodoterullarubra	5	13.89	0	0.00
Zygosaccharomyces spp.	2	5.56	0	0.00
Total	**36**	**100.00**	**8**	**100.00**

Results of identified molds in the examined samples of rayeb milk:

Table (3): Frequency distribution of identified molds in examined samples of rayeb milk

Isolates of molds	Plain rayeb (n=20)		Sterilized rayeb (n=20)	
	No.	%	No.	%
Aspergillus flavus	9	10.84	1	5.88
Aspergillus niger	11	13.25	3	17.65
Alternaria alternate	10	12.05	2	11.76
Cladosporium clavocipus	7	8.43	2	11.76
Helminthosporium spp.	3	3.61	2	11.76
Penicillium chrysogenum	9	10.84	5	29.41
Penicillium roqueforti	12	14.46	0	0.00
Penicillium rubrum	9	10.84	0	0.00
Rhizopous spp.	6	7.23	1	5.88
Mucor spp.	2	2.41	1	5.88
Acremonium spp.	5	6.02	0	0.00
Total	**83**	**100.00**	**17**	**100.00**

Results of yeasts and molds counts (CFU/ml) in the examined samples of yoghurt:

Table (4): Statistical analytical results of yeasts and molds counts (CFU/ml) in yoghurt samples

Yoghurt samples	CFU/g	Positive samples		Minimum	Maximum	Mean ± SEM
		No.	%			
Plain (n=40)	Yeasts	40	100.00	7.32×10^{3}	9.41×10^{3}	$8.37\times 10^{3} \pm 2.40 \times 10^{2a}$
	Molds	40	100.00	4.12×10^{3}	3.27×10^{3}	$3.09\times 10^{3} \pm 1.50 \times 10^{2a}$
Sterilized Strawberry (n=20)	Yeasts	20	100.00	6.14×10^{3}	8.32×10^{3}	$7.23\times 10^{3} \pm 2.55 \times 10^{2b}$
	Molds	20	100.00	3.35×10^{3}	4.30×10^{3}	$3.83\times 10^{3} \pm 1.77 \times 10^{2c}$
Sterilized Peach (n=20)	Yeasts	20	100.00	6.55×10^{3}	8.25×10^{3}	$7.40\times 10^{3} \pm 3.52 \times 10^{2b}$
	Molds	20	100.00	3.31×10^{3}	3.53×10^{3}	$3.42\times 10^{3} \pm 3.41 \times 10^{2c}$

Values are three independent determination. Mean values having different superscript letters in a column for each sample are significantly different ($P \leq 0.05$).

Results of identified yeasts in the examined yoghurt samples:

Table (5): Frequency distribution of identified yeasts in examined samples of yoghurt

Isolated of yeasts	Plain yoghurt (n=40)		Sterilized yoghurt (n=40)			
			Strawberry (n=20)		Peach (n=20)	
	No.	%	No.	%	No.	%
Candida albicans	8	16.33	4	22.22	5	22.73
Candida krusei	5	10.20	5	27.78	6	27.27
Candida tropicalis	3	6.12	2	11.11	3	13.64
Candida parapsillosis	5	10.20	3	16.67	5	22.73
Candida stellotiodes	9	18.37	0	0.00	0	0.00
Candida pseudotropicalis	5	10.20	0	0.00	0	0.00
Rhodoterullaglutinis	3	6.12	4	22.22	3	13.64
RhodoterullaPichia	5	10.20	0	0.00	0	0.00
Rhodoterullarubra	4	8.16	0	0.00	0	0.00
Zygosaccharomyces spp.	2	4.08	0	0.00	0	0.00
Total	**49**	**100.00**	**18**	**100.00**	**22**	**100.00**

Results of identified molds in the examined yoghurt samples:

Table (6): Frequency distribution of identified molds in examined samples of yoghurt

Isolates of molds	Plain yoghurt (n=40)		Sterilized yoghurt (n=40)			
			Strawberry (n=20)		Peach (n=20)	
	No.	%	No.	%	No.	%
Aspergillu sniger	12	12.00	4	20.00	3	10.34
Aspergillus flavus	9	9.00	2	10.00	3	10.34
Aspergillus parasiticus	3	3.00	0	0.00	1	3.45
Penicillium chrysogenum	15	15.00	0	0.00	0	0.00
Penicilliumrubrum	13	13.00	7	35.00	9	31.03
Fusarium spp.	0	0.00	2	10.00	0	0.00
Alternaria alternate	14	14.00	2	10.00	8	27.59
Cladosporium clavocipus	6	6.00	3	15.00	5	17.24
Helminthosporium spp.	11	11.00	0	0.00	0	0.00
Rhizopous spp.	5	5.00	0	0.00	0	0.00
Mucor spp.	4	4.00	0	0.00	0	0.00
Acremonium spp.	8	8.00	0	0.00	0	0.00
Total	**100**	**100.00**	**20**	**100.00**	**29**	**100.00**

Results of mycological hygienic quality of the totally examined fermented milk product samples:

Table (7): Summarized results of mycological examination of fermented milk products samples in compared with the Egyptian standards (EOSQC, 2005)

Counts	Egyptian Standards	Plain rayeb (n=20)				Sterilized rayeb (n=20)				Plain yoghurt (n=40)				Sterilized Strawberry yoghurt (n=20)				Sterilized Peach yoghurt (n=20)			
		Acceptable		Unacceptable		Acceptable		Unacceptable		Acceptable		Unacceptable		Acceptable		Unacceptable		Acceptable		Unacceptable	
		No.	%	No	%	No	%	No	%	No	%	No	%	No.	%	No	%	No	%	No	%
Yeasts and molds	Not more than 10/g	0	0	20	100	0	0	20	100	0	0	40	100	0	0	20	100	0	0	20	100

Acceptable: means that the examined samples were within the permissible limit of the Egyptian Standards.
Unacceptable: means that the examined samples failed to follow the Egyptian Standards.

Results of Aflatoxin M_1 in rayeb milk and yoghurt determined by ELISA:

Table (8): Estimation of Aflatoxin M_1 in the examined fermented milk samples by Enzyme Linked Immunoasorbant Assay (ELISA)

Samples (n = 3)			Aflatoxin M_1 (mg/kg)
Rayeb	Plain		11.0 ± 1.39^{b}
	Sterilized		0.020 ± 0.003^{d}
Yoghurt	Plain		20.0 ± 3.69^{a}
	Sterilized	Strawberry	0.042 ± 0.006^{c}
		Peach	0.007 ± 0.0001^{e}

Mean values having different superscript letters in a column for each sample are significantly different ($P \leq 0.05$).

4. Discussion:

Presence of yeasts and mold in milk and dairy products are undesirable even when found in few numbers as they resulting in objectionable changes that render the products of inferior quality **(Abd El-Hameed, 2011).** Yeasts and molds growing in yoghurt utilized some of the acid and produce a corresponding decrease in the acidity, which may favor the growth of putrefactive bacteria **(Oyeleke, 2009).**

According to the recorded data in **Table (1),** it was found that yeasts and molds organisms were detected at an incidence of 100 % in all of the examined samples of plain and sterilized rayeb. Also, it was recorded that the mean value of yeasts count was $8.12 \times 10^5 \pm 3.10 \times 10^2$ and $7.04 \times 10^5 \pm 2.13 \times 10^2$ CFU/ml for plain and sterilized rayeb, respectively.

On the other hand, the mean value of molds count was $4.45 \times 10^5 \pm 2.14 \times 10^2$ and $3.53 \times 10^5 \pm 2.45 \times 10^2$ CFU/ml for plain and sterilized rayeb, respectively. Statistical analysis of the obtained results clarified that there was a significant difference ($P \leq 0.05$) in mean values of yeasts and molds counts of the examined samples of plain and sterilized rayeb and means values of yeast count was higher that mean values of molds count. Generally, it was clear that plain rayeb was more contaminated than sterilized rayeb by yeasts and molds. The recorded results were in harmony with **Ahmed (1995)** ($3.15 \times 10^5 \pm 1.2 \times 10^3$ CFU/g), **Riad (1996)** ($3.20 \times 10 \pm 1.77 \times 10$ CFU/g), **Adebesin *et al.* (2001)** who recorded that yeasts counts were from 0.0 $\times 10^4$ CFU/ml and to 5.3 $\times 10^4$ CFU/ml in locally manufactured fermented milk (fura and da nono) in

Nigeria, **Abdel All and Dardir (2009)** who recorded that the total yeasts and mold count ranged from 1.6×10 to 10 CFU/g, **El-Malt *et al.* (2013)** who could detect yeasts in 94% with average counts of 1.4 ×10 CFU/g for small scale and 40% with average counts 3.9 ×10 CFU/g of large scale yoghurt samples. **El-Ansary (2014)** who found that yeasts and mold counts $5.6 \times 10^4 \pm 4.9 \times 10$ and $5.3 \times 10^4 \pm 3.33 \times 10^3$, respectively and **Dirisu *et al.* (2015)** who found that total fungi count was ranged from 3.2×10^3 to 4.9×10^3 CFU/ml. According to the Egyptian Standards (Yeasts and molds must be not more than 10 /g), it was noticed that all of the examined samples of plain and sterilized rayeb failed to comply with the Egyptian limits **(Table, 7).**

Presented data in **Table (2)** showed the frequency distribution of identified yeasts recovered from the examined samples of rayeb milk. It was noticed that*Candida albicans, Candida krusei, Candida tropicalis, Candida stellotiodes, Candida guillormondi, Candida parapsillosis, Candida pseudotropicalis, Rhodoterullaglutinis, Rhodoterulla pichia, Rhodoterullarubra* and *Zygosaccharomyces spp.* were isolated at different frequencies from the examined samples of plain rayeb (36 isolates) where *Rhodoterullaglutinis*was the highest isolated *(*19.44%) followed by *Candida albicans*(16.67%) while *Candida guillormondi, Candida pseudotropicalis* and *Rhodoterulla pichia* were the lowest isolated (2.78% of each). Concerning sterilized rayeb samples, it was noticed that *Candida albicans, Candida krusei, Candida parapsillosis, Candida pseudotropicalis* and *Rhodoterullaglutinis* were isolated at different frequencies from the examined samples (8 isolates) where *Rhodoterullaglutinis* was the highest isolated (37.5%) followed by *Candida albicans* (25%).

Identified molds from the examined samples of rayeb milk were tabulated in **Table (3).** The frequency distribution of identified molds in examined samples of plain rayeb showed that *Penicilliumroqueforti*was the highest isolated mold (14.46%) followed by *Aspergillusniger* (13.25%) then *Alternaria alternate* (12.05%) and *Aspergillus flavus, Penicillium chrysogenum and Penicilliumrubrum* (10.84% of each) While *Mucor spp.* were the lowest isolated (2.41%). Concerning sterilized rayeb samples, it was noticed that *Penicillium chrysogenum* was the highest isolated (29.41%) followed by *Aspergillus niger* (17.65%) then *Alternaria alternate, Cladosporiumclavocipus* and*Helminthosporium spp.* (11.76% of each) and lastly *Aspergillusflavus, Rhizopous spp. and Mucor spp.* (5.88% of each).

Results of yeasts and molds counts (CFU/ml)in the examined samples of yoghurt were recorded in **Table (4).** It was found that the mean values of yeasts count in the examined samples of plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt were$8.37 \times 10^5 \pm 2.4 \times 10^2$, $7.23 \times 10^5 \pm 2.55 \times 10^2$ and $7.40 \times 10^5 \pm 3.52 \times 10^2$, respectively. On the other hand, the mean values of molds count in the examined samples of plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt were $3.09 \times 10^5 \pm 1.5 \times 10^2$, $3.83 \times 10^5 \pm 1.77 \times 10^2$ and 3.42 $\times 10^5 \pm 3.41 \times 10^2$. Statistical analysis of the obtained results clarified that there was a significant difference (P≤0.05) in mean values of yeasts and molds counts of the examined samples of plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt and means values of yeast count was higher that mean values of molds count**.** Generally, it was clear that yeasts and molds counts of both plain and sterilized yoghurt were nearly equal that may throw the light on doubtful effeciecy of sterilization process or post manufacture contamination during transportation, handling and storage of retailed yoghurt**.** According to the Egyptian Standards(Yeasts and molds must be not more than 10 /g), it was noticed that all of the examined samples of plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt failed to comply with the Egyptian limits **(Table, 7).**

The legal standard of yeasts cells in produced yoghurt under conditions of good manufacturing practices should be not more than 10 yeasts cells in 3-4 weeks shelf life yoghurt at 5° C. However, yeasty and fermented off-flavors and gassy appearances are often detected when yeasts grow to 10^5-10^6 CFU/g **(Ledenbach and Marshall, 2007).**

Identified yeasts in the examined yoghurt samples were tabulated in **Table (5).** The frequency distribution of identified yeasts in examined samples of plain yoghurt revealed that *Candida stellotiodes* was the highest isolated (18.37%) followed by *Candida albicans* (16.33%), then *Candida krusei, Candida parapsillosis, Candida pseudotropicalis*and *Rhodoterulla pichia* (10.20% of each) while *Zygosaccharomyces spp.* were the lowest isolated (4.08%). On the other side, the frequency distribution of identified yeasts in examined samples of sterilized yoghurt revealed that *Candida krusei* was the highest isolated from both strawberry and peach yoghurt (27.78 and 27.27%, respectively) followed by *Candida albicans* (22.22 and 22.73%, respectively).

Identified molds in the examined yoghurt samples were illustrated in **Table (6).** The frequency distribution of identified molds in examined samples of plain yoghurt revealed that *Penicilliumchrysogenum*was the highest isolated (15 %) followed by *Alternaria alternate* (14%) then *Penicillium rubrum*(13%), *Aspergillu sniger* (12%) while *Mucor spp.* were the lowest isolated(4%). On the other side, the frequency distribution of identified molds in examined samples of sterilized strawberry yoghurt revealed that *Penicillium rubrum*was the highest isolated (35%) followed by *Aspergillus niger* (20%) then *Cladosporium clavocipus* (15%), *Aspergillu sflavus, Fusarium spp.* And *Alternaria alternate* (10% of each). Concerning sterilized peach yoghurt, *Penicilliumrubrum* was the highest isolated (31.03%) followed by *Alternaria alternate* (27.59%) and *Cladosporium clavocipus*(17.24%) then *Aspergillus niger* and *Aspergillus flavus* (10.34%) and lastly *Aspergillus parasiticus* (3.45 %).

The obtained results were in agreement with **Shawer (1997)** who observed that the most prevalent isolates of yeasts and mold were *Candida, Rhodotorula torulopsis* and *Sccharomyces spp*. While, the most prevalent mold isolates were *Aspergillus, Alternaria*, *Penicillium* and *Cladosporium spp*. and **Adebesin *et al.* (2001)** who could isolate *Aspergillus flavus* and *Rhizopusnigricans* from the locally manufactured fermented dairy products.

Human food can be contaminated with mycotoxins at various stages in the food chain and the most important genera of mycotoxigenic fungi are *Aspergillus*, *Alternaria*, *Claviceps*, *Fusarium*, *Penicillium* and *Stachybotrys*. The principal classes of mycotoxins include a metabolite of *Aspergillusflavus and Aspergillusparasiticus* **(Bennett and Klich, 2003)**. Mortality was high and death occurred suddenly as a result of massive gastrointestinal hemorrhage **(Abbas *et al.,* 2004).** Mycotoxins are organic mixes delivered by molds that can influence nourishment quality and assembling. In this manner, for the customers' wellbeing, it is important to distinguish the nearness and measure of mycotoxins in sustenance industriously and plan to minimize them in the evolved way of food chain **(Norian *et al.,* 2015).**

Results of estimation of Aflatoxin M_1 in the examined fermented milk samples by enzyme immunoassay (ELISA) were recorded in **Table (8).** It was recorded that Aflatoxin M_1 was determined in plain and sterilized rayeb at the rate of 11.0 ± 1.39 and 0.020 ±0.003 mg/kg, respectively. While Aflatoxin M_1 was determined in plain yoghurt, sterilized strawberry yoghurt and sterilized peach yoghurt at the rate of 20.0 ± 3.69, 0.042 ± 0.006 and 0.007 ± 0.0001 mg/kg, respectively. Statistical analysis of the obtained results clarified that there was a significant difference ($P \leq 0.05$) in mean values estimation of Aflatoxin M_1.

The obtained results were in agreement with **Issazadeh *et al.* (2012)** who examined 60 samples of local yogurt in Gilan (Northern Iran) with ELISA technique to determine the presence and the level of AFM_1 and found that 98.33% had AFM_1 in concentrations between 6.2 - 87ng/l and the mean level of AFM_1 of positive samples was 51.66 ng/l, **Iha *et al.* (2013)** who investigated the occurrence of AFM in Brazilian milk and found that AFM was detected in 83% of the milk samples (>3 ng/kg) with levels ranging 1 from 8 to 437 ng/kg for fluid milk, and 20 to760 ng/kg for powdered milk. Also, they found that processing and storage was shown to have little effect on AFM content in milk and milk products and the total AFM mass in milk was reduced by 3.2% in cheese and by 6% in yoghurt (pH 4.4) and **Kos *et al.* (2014)** who investigated the occurrence of AFM1 in 150 cow's using ELISA method and found that AFM1 was detected in 98.7% of analyzed cow's milk samples in concentrations ranged from 0.01 to 1.2 µg/kg. Further, even 129 (86.0%) cow's milk samples contained AFM1 in concentration greater than maximum residue levels (MRL) of 0.05 µg/kg defined by European Union (EU) Regulation although, having levels under this limit does not mean that it is safe and the countries tended to decrease the limit (**Taydaş and Aşkın, 1995).**

Detection of Aflatoxin M_1 in the examined samples highlighted the significance of fermented dairy products as source of mycotoxin to consumers that was supported by **Atanda (2007)** and **Sun *et al.* (2011)** who reported that Aflatoxins were ubiquitous foodborne toxicants and the co-occurrence of these mycotoxins in foods represents a significant public health concern, which was strongly associated with human aflatoxicosis, neural tube defects and many types of primary cancers. The chronic exposure may also lead to high risk of developing liver cancer, as aflatoxin metabolites can intercalate into DNA and Alkylate the bases through its epoxide moiety. Infection by hepatitis B virus (HBV) and/or HCV and dietary exposure to aflatoxin are the main risk factor for the development of chronic liver disease and hepatocellular carcinoma **(Lereau *et al.,* 2012).** High concentration of Aflatoxin in milk and milk products causes widespread negative impact on public health. Therefore, it is necessary to establish strategies for reducing aflatoxin levels in animal feed and milk products.

Conclusion:

In conclusion, the current study reported the detection of potentially important fungi and their mycotoxins of zoonotic importance in some fermented milk products. Also in light of the above, rayeb and yoghurt samples obtained from some Alexandria markets constitute a high risk health hazard to consumers. The finding of the study warrant the need to undertake safety measures as HACCP system (Hazard analysis and critical control point) to avoid potential threats and apply educational programs for dairy products producers about the risk of the contamination of this product, how to prevent or at least reduce this pathogens from the yoghurt and how to apply strict hygienic measures during production, storage and distribution of the rayebmilk and yoghurt. Moreover regulation of small scale producing rayeb milk and yoghurt in Alexandria should be a part of a strategy to enhance producing of save and high quality dairy products.

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