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Microbial Profile of Heat-Treated Milk Sold at Local Markets

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ABSTRACT

A total of one hundred random samples of heat treated milk (pasteurized and UHT, 50 of each) were collected randomly from Groceries and super markets at local markets in Alexandria Governorates for chemical, sanitary and microbiological evaluation. Mean values of fat, protein, lactose, SNF and minerals of examined pasteurized and sterilized (UHT) milk were (2.5±0.16, 3.3±0.6), (3.3±0.07, 3.4±0.07), (5.4±0.25, 5.7±0.27), (8.7±0.16, 9.07±0.25) and (0.51±0, 02, 0.54±0.02), respectively. Sanitary evaluation of examined pasteurized and UHT milk samples was revealed that, the mean values of titratable acidity and pH values were (0.15±0.0025, 0.14±0.002) and (6.66±0.06, 6.65±0.03) respectively. Microbiological evaluation revealed that the mean values of total bacterial count in examined pasteurized and sterilized (UHT) milk samples were (6.9 $x10^2$±0.74 $x10^2$ and 4.7 $x10^2$±0.73 $x10^2$), respectively. On the other hand, total Coliform and faecal Coliforms count were found with mean values of (5.7 $x10^2$±0.6 $x10^2$ and 1.5 $x10^2$±0.45 $x10^2$) for Coliform organisms while, (8.0 x10±0.45 x10 and 4.5 x10±1.5 x10) for faecal Coliforms, respectively. Entero Pathogenic E.coli organisms were failed to be detected in all examined pasteurized and sterilized milk samples. Clostridia organisms were detected in a percent of 14 and 12% in examined pasteurized and UHT milk samples with a mean values of (2.4 x10±0.57x10 and 3.8 x10±0.83 x10) respectively. The most prevalent isolated clostridia species were Clostridium Chauvoei, Clostridium septicum, Clostridium novyi, Clostridium perfringens with variable percent of (2, 3, 3, 0 and 4, 3, 3, 1) in both examined pasteurized and UHT milk samples, respectively. Mean values of isolated yeast count from pasteurized and UHT milk samples were (1.7x10±0.3 x10 and 1.3x10±0.2x10) with incidence percent of 12% in both samples, respectively. On the other hand, the mean values of mould count were (2.8x10±0.24x10 and 7.5x10±0.97x10), respectively. Incidence percent of mould contamination was 84 and 88% in both examined pasteurized and UHT milk samples. On the other all the prophylactic hygienic measures should be undertaken to safe guard the consumer health and prevent contamination of heat treated milk.

1. INTRODUCTION

Heat treatment of the produced milk may safeguard consumer from being infected with various pathogens. But in fact acts as a common type of adulteration as it covers the unsanitary conditions under which milk is produced, as well as, increasing the keeping quality of milk (Mansour et al.2007).

Pasteurization is a process designed to destroy non-pathogenic microorganisms of raw milk, two-steps process consists of rapid heating of the milk followed by immediate cooling. Moreover, this procedure is applied to obtain milk that is fit for human consumption and has longer conservation time (Kameni et al. 2002). Pasteurization as a practice has a positive effect on the bacteriological contents of milk, as it reduces the total bacterial count (TBC), Coliform bacterial count and other pathogens (El Zubeir et al. 2007).

According to the international Dairy Federation (IDF), pasteurization is defined as a process applied to a product with the object of minimizing possible health hazards arising from pathogenic microorganisms associated with milk by heat treatment; it is consistent with minimal chemical, physical and organoleptic changes in the product (Ledenbach and Marshal, 2009).

UHT milk has gained increased acceptance during the last few years mainly due to its convenience to the Egyptian hot weather and as a method of storage of fresh raw milk and the possibility to produce UHT milk from recombined milk (Abo-Donia et al. 1985).

Pasteurized milk is recommended to be consumed within seven days from the production while UHT milk is for six months from the production date. Poor initial milk quality, faulty processing, problem in preservation introduction at the consumer side may lead to microbial contamination in milk and give a great chances of milk deterioration prior than the recommended preservation time (BSTI, 2002).

High bacterial count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination due to poor processing and handling conditions and/or poor hygienic practices by workers. According to the definition of UHT process, UHT milk should contain very little or no active bacteria (Hassan et al. 2009).

The presence of Coliform organisms in milk and milk products such as pasteurized and UHT milk give an indication about sanitary production and\or improper handling of either milk or milk utensils that may leads to deterioration of the product and induce public health hazards (El-Zubeir and Ahmed, 2007).

Pasteurized milk shouldn't contain any coliform bacteria as though coliform bacteria can't survive the pasteurization temperature but the presence of Total coliform count of the pasteurized milk samples indicates either defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials (Srairi et al. 2006).

The most important spore forming organism is Clostridium perfringens which produce toxins associated to the spore forming process especially or due to result of heat resistance and causing public health hazards (Torres-Anjel and Butcher, 1980).

Fungal contamination of heat treated milk mainly UHT milk is indicative for errors in storage and defects in packaging processes. Moreover, multiplication of these fungi in UHT milk during storage may induce undesirable flavour, poor appearance and discoloration (Bullerman, 1981).

The public health importance of mould lies in that certain species are mycotoxins producers, even at temperatures as low as -2 to 10^{o}C and have been implicated in cases of human food poisoning and neoplastic diseases. Some species of yeasts as Candida species constitute a public health hazard (Bullerman, 1980).

Aflatoxins are main secondary metabolites of fungi. These metabolites are produced by Aspergillus flavus, A. parasiticus and A. nomius. After the ingestion of AFB1 by dairy cattle, AFB1 is transformed to a hydroxylated derivative in the liver, namely Aflatoxin M1 (AFM1) (Aycicek et al. 2005).

The present study was conducted to evaluate the quality of pasteurized and UHT milk sold at local markets in Alexandria city through evaluation of chemical composition, sanitary and microbiological quality.

2. MATERIAL AND METHODS

1. Collection of samples:

A total of one hundred samples of heat-treated milk samples including (50 of pasteurized and 50 of UHT milk) were collected randomly from groceries and supermarkets at local markets in Alexandria Governorate.

2. Chemical examination of raw milk using Milk Scan (Lacto-scan).

3. Sanitary evaluation of examined raw milk:

3.1. Determination of titratable acidity percent according to (AOAC, 2005).

3.2. Determination of pH value using pH meter according to (AOAC, 2005).

4. Microbiological evaluation of heat treated milk samples:

4.1. Preparation of serial dilution (APHA, 1992).

One ml of well mixed milk sample was added separately to 9 ml of sterile saline solution then thoroughly mixing for preparation of 10-fold serial dilutions, from which the decimal dilutions were prepared.

4.2. Total aerobic plate count (APHA, 1992).

4.3. Coliforms count (APHA, 1985).

4.4. Anaerobic spore former count (Gudkov and Sharpe, 1966).

4.5. Total Yeasts and total moulds count (Bailey and Scott, 1998).

4.6. Estimation of Aflatoxins produced by toxigenic strains of Aspergillus flavus.

3. RESULTS AND DISCUSSION

Table (1): statistical analytical results of chemical composition of examined pasteurized and UHT milk samples.

Parameters	Pasteurized milk	UHT milk
Fat	2.5±0.16	3.3±0.06
Protein	3.3±0.07	3.4±0.07
Lactose	5.4±0.25	5.7±0.27
SNF	8.7±0.16	9.07±0.25
Minerals	0.51±0.02	0.54±0.02

SNF: Sold not fat

Table (2): Comparison between the obtained results of chemical composition of examined pasteurized and UHT milk samples with Egyptian Standards **(ES, 154/2 and 3/2005)**.

Parameters	Fat %				SNF %			
	Compatible samples		Incompatible samples		Compatible samples		Incompatible samples	
	No.	%	No.	%	No.	%	No.	%
Pasteurized milk	45	90	5	10	47	94	3	6
UHT milk	50	100	0	0	47	94	3	6
Egyptian standards, 2005	**Not less than 3**				**Not less than 8.25**			

Table (3): Titratable acidity and pH value of examined pasteurized and UHT milk samples.

Parameters	Range	Pasteurized milk	Range	UHT milk
Acidity percent	0.12-0.18	0.15± 0.002	0.11-0.18	0.14 ± 0.002
pH value	6.35-6.87	6.66± 0.06	6.50-6.85	6.65 ± 0.03

Table (4): Comparison between the obtained results of acidity percent of examined pasteurized and UHT milk samples with Egyptian Standards (ES, 154/2/2005 for pasteurized milk and 154/3/2005 for UHT milk).

	No. of examined samples	Compatible samples		incompatible samples	
		No.	%	No.	%
Pasteurized milk	**50**	48	96	2	4
UHT milk	**50**	49	98	1	2
Egyptian standards	**Acidity percent not more 0.17**				

Table (5): Microbiological evaluation of examined pasteurized and UHT milk samples.

	Pasteurized milk			UHT milk		
	No. of positive samples	**%**	**Mean ± SEM**	**No. of positive samples**	**%**	**Mean ± SEM**
TBC	15	30	$6.9x10^2 \pm 0.74x10^2$	10	20	$4.7x10^2 \pm 0.73x10^2$
Coliform	7	14	$5.7x10^2 \pm 0.6x10^2$	5	10	$1.5x10^2 \pm 0.45x10^2$
F. Coliforms	3	6	8.0x10 ± 1.5x10	2	4	4.5x10 ± 1.5x10
Clostridial count	7	14	2.4x10 ± 0.57x10	6	12	3.8x10 ± 0.83x10
Yeast	6	12	1.7x10 ± 0.3x10	6	12	1.3x10 ± 0.21x10
Mould	4	8	2.4x10 ± 0.57x10	3	6	7.5x10 ± 0.97x10

F:feacal coliform

Table (6): Comparison between the microbiological evaluations of examined heat treat milk samples with Egyptian Standards **(ES, 154/2/2005 for pasteurized and154/3/2005 for UHT milk).**

	Pasteurized milk (n=50)				UHT milk (n=50)			
Parameters	**Compatible samples**		**Incompatible samples**		**Compatible samples**		**Incompatible samples**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Total bacterial count	50	100	0	0	40	80	10	20
Egyptian standards, 2005	**Not more than 30.000 cells /ml**				**Not more than 10 cells/ml**			
Coliforms	43	86	7	14	45	90	5	10
	Not more than 10 cells /ml				**Nil**			

Table (7) Statistical analytical results of Clostridial organisms count (cfu/ml) in examined pasteuized and UHT milk samples.

Products	**Positive sample**		**Clostridial count**		
	No	**%**	**Minimum**	**Maximum**	**Mean ± SEM**
Pasteurized milk	**7**	**14**	1.0x10	5.0x10	2.4x10 ± 0.57x10
UHT milk	**6**	**12**	1.0x10	7.0x10	3.8x10 ± 0.83x10

SEM= Standard Error of Mean

Table (8): Frequency distribution of isolated clostridia species in the examined pasteurized and UHT milk samples

Types	**Pasteurized milk**		**UHT milk**	
	No. of isolates	**%**	**No. of isolates**	**%**
Clostridium Chauvoei	2	25	4	36.37
Clostridium Septicum	3	37.5	3	27.27
Clostridium Novyi	3	37.5	3	27.27
Clostridium Perfringens	0	0	1	9.09
Total	8	100	11	100

Table (9): Frequency distribution of identified yeasts isolated from examined pasteurized and UHT milk samples.

Identified yeasts spe	Pasteurized milk		UHT milk	
	No	%	No	%
Candida albicans	3	20	5	29.41
Candida krusei	3	20	3	17.64
Candida pseudotropicalis	3	20	3	17.64
Candida tropicalis	2	13.33	2	11.77
Rhodotorullaglutinis	2	13.33	2	11.77
Rhodotorullarubra	2	13.33	2	11.77
Total	15	100	17	100

Table (10): Frequency distribution of identified mould isolated from examined pasteurized and UHT milk samples

Identified moulds	Pasteurized milk		UHT milk	
	No	%	No	%
Aspergillus flavus	2	20	1	12.5
Alternaria alternata	-	-	2	25
Aspergillus fumigatus	2	20	2	25
Aspergillus niger	1	10	2	12.5
Cladosporium clavocipus	2	20	-	-
Penicillium citrinum	2	20	1	12.5
Rhizopous spp.	1	10	1	12.5
Total	10	100	8	100

Table (11): Aflatoxin B1 concentrations produced by Aflatoxigenic strain of Aspergillus flavus isolated from heat treated milk samples.

Asp.flavus	Source of milk	Aflatoxine B1 Conc.(ppb)
Strain 1	Pasteurized	0.67
Strain 2	Pasteurized	0.92
Strain 3	UHT	0.79

3.1. Chemical evaluation of examined heat treated milk:

Table (1) showed that the mean values of chemical parameters of examined pasteurized and UHT milk samples were (2.5±0.16 and 3.3±0.06) for fat, (3.3±0.07 and 3.4±0.07) for protein, (5.4±0.25 and 5.7±0.27) for lactose, (8.7±0.16 and 9.07±0.25) for SNF and (0.51±0.02 and 0.54±0.02), for mineral content, respectively.

The protein content of cow's milk has been reported to vary from 3.22 to 3.92 % (Lingathurai et al., 2009). (Saha and Ara, 2012) reported that The protein contents of the five pasteurized brands milk (above 4%) were within the acceptable limit according to (BSTI 2002) standard (not lower than 3.3%). The total nitrogen tended to decrease while the non–protein nitrogen (NPN) and non–casein nitrogen (NCN) fractions tended to increase during storage (Mehanna and Gonc, 1988).

Egyptian Standards (154/2, 3/2005) stated that fat percent and SNF of pasteurized and UHT milk samples should be not less than 3 and 8.25 %, respectively according to this standard, 90 and 100 % of examined pasteurized and UHT milk samples were compatible with fat standard and 94 % of both examined pasteurized and UHT milk samples were compatible with SNF standard Table (2).

3.2. Sanitary evaluation of examined heat treated milk samples: Table (3) revealed that the mean values of titratable acidity in examined pasteurized and UHT milk samples were (0.15± 0.002 and 0.14 ± 0.002), respectively. While the mean values of pH values in examined pasteurized and UHT milk samples were (6.66±0.06 and 6.65 ± 0.03), respectively.

Egyptian Standards (2005) stated that acidity percent of pasteurized and UHT milk should not more than 0.17 %, according to this standard, 96 and 98 % of examined pasteurized and UHT milk were compatible with standard, while, 4 and 2 % of examined pasteurized and UHT milk samples were incompatible with acidity standard table (4).

Our results of titratable acidity for UHT milk agreed with the results that reported by (Hossain et al. 2011) who find that the average of initial acidity percentage for UHT milk samples was 0.145±0.011; this emphasize two possibilities that the initial high acidity may have developed prior to the heat treatment and in the same time improper heat treatment may results into presence of bacterial population in treated milk which might be also results into high initial acidity in UHT milk.

PH value is an important indicator to test milk quality (Goff, 2009). Some studies mentioned that knowledge of the initial pH prior to heating alone was not sufficient for predicating the changes that occurred during heating and storage (Chandrapala et al. 2010). The pH level was decreases with prolonged storage period. Also, the results showed that a reverse correlation between acidity level and pH in milk, when acidity level increases pH level decreases. (Farkye et al. 2001). (Clare et al. 2005) concluded that when the storage period was prolonged the acidity and bacterial counts were increased while pH decreased.

3. Microbiological evaluation of examined heat treated milk sample: Data presented in Table (5) showed that the incidence of total bacterial count, Coliforms, faecal Coliform, Clostridial count and yeast and mould in examined pasteurized milk samples were 30, 14, 6, 14, 12 and 8 % respectively, with mean value of $6.9x10^2$ ± $0.74x10^2$,$5.7x10^2$ ± $0.6x10^2$,8.0x10 ± 1.5x10, 2.4x10 ±0.57x10, 1.7x10± 0.3x10 and 2.8x10 ± 0.24x10, respectively. on the other hand, the incidence of total bacterial count, Coliforms, faecal Coliform, Clostridial count and yeast and mould in examined UHT milk samples were 20, 10, 4, 12, 12 and 6 % respectively, with mean value of $4.7x10^2$ ± $0.73x10^2$, $1.5x10^2$ ± 0.45x10, 4.5x10±1.5x10, 3.8x10± 0.83x10, 1.3x10± 0.21x10, and 7.5x10±0.97x10, respectively.

Egyptian Standards (2005) stated that total bacterial count of pasteurized and UHT milk samples should not more than 30.000 and 10 cells /ml, respectively, according to this standard, all examined pasteurized milk samples were compatible with standard, while 80 % of UHT milk samples were compatible with Egyptian standard table (6).

Egyptian Standards (2005) stated that coliforms count in pasteurized should not more than 10 cells/ml, according to this standard, 86% of examined pasteurized milk was compatible with standard and 14 % were incompatible with this standard. On the other hand, UHT milk must be free from Coliforms organisms, according to this standard 90% of examined UHT milk samples were compatible with standard and 10 % were incompatible with standard (Table, 6).

Occurrence of bacterial contamination in pasteurized milk was due to defect in pasteurization machinery, surviving the bacteria after pasteurization, contamination in the post-pasteurized process due to poor processing and handling conditions and/or maintenance of substandard hygienic practices by working personnel. (Sourav et al. 2014).

Coliform bacteria are supposed to be absent in pasteurized milk as they can't survive the pasteurization temperature. Because of the defect in pasteurization process or post pasteurization contamination which includes contamination in packaging materials, defects in pipe lines, Total Coliform count may be detected in the pasteurized milk samples (Dey and Karim, 2013).

Escherichia coliorganisms were failed to be detected in pasteurized and sterilized milk sample over results

The presence of coliform organisms in UHT milk is considered as bad index for the lower hygienic quality and leads to deterioration of the product and causing public health hazards (Saudi et al. 1990).

The obtaining results in table (7) showed the incidence of clostridia organism in pasteurized and UHT milk samples were 14 and 12 %, respectively with a mean values of 2.4x10± 0.57x10 and 3.8x10± 0.83x10 and arange of 1.1x10 to5.0x10 and 1.0x10 to 7.0x10, respectively.

The data presented in table (8) showed that the most prevalent clostridia species that could be isolated from examined pasteurized milk samples were identified as Clostridium chauvoei 2(25%),Clostridium Septicum 3(37.5%),Clostridium Novyi 3(37.5%) and Clostridium Perfringens 0, while the most prevalentclostridium species isolated from UHT milk samples were Clostridium Chauvoei

4(36.37%), Clostridium Septicum 3(27.27%), Clostridium Novyi 3(27.27%) and Clostridium Perfringens 1(9.09%), respectively.

The data presented in Table (9) showed that the most prevalent yeast that could be isolated from examined pasteurized milk samples were identified as Candida albicans 3 (20%), Candida krusei 3(20%), Candida pseudotropicalis 3(20%), Candida tropicalis 2 (13.33%), Rhodotorulla glutinis 2(13.33%) and Rhodotorula rubra 2(13.33%), respectively. While, most prevalent yeast species that could be isolated from examined UHT milk samples were identified as Candida albicans 5 (29.41%), Candida krusei 3 (17.64%), Candida pseudotropicalis 3 (17.64%), Candida tropicalis 2 (11.77%), Rhodotorulla glutinis 2 (11.77%) and Rhodotorula rubra 2 (11.77%), respectively.

Our results disagree with (Al-Tahiri, 2005) who stated that the rate of detection yeast and mould in UHT milk samples was zero.

Yeasts organisms occur in raw and pasteurized milks at low populations, generally $<lo^3$ cells per milliliter. These yeasts rarely grow in milk during refrigerated storage, and are quickly overgrown by Psychrotrophic However, in the absence of significant bacterial competition; yeasts can establish very good growth in milk. Thus, several species of Candida, KLuyveromyces marxianus, Cryptococcus flavus, and Saccharomyces cerevisiae readily grew to populations of lo^8 to lo^9 cells per milliliter after inoculation into sterilized milk (Fleet, 1990).

Some species of yeasts as Candida species constitute a public health hazard as they may cause endocarditis and occasionally some fatal systemic diseases (Jacquet and Teherani, 1976).

The data presented in Table (10) showed that the most prevalent moulds species that could be isolated from examined pasteurized milk samples were identified as Aspergillus flavus 2 (20%), Aspergillus Fumigatus 2(20%), Aspergillus Niger 1 (10%), Cladosporium Clavocipus 2 (20%), Penicillium citrinum 2(20%) and Rhizopous species 1(10%), respectively. While, the most prevalent moulds species that could be isolated from examined UHT milk samples were identified as Aspergillus flavus 1(12.5%), Alternaria alternata 2 (25 %), Aspergillus Fumigatus 2(25%), Aspergillus Niger 1(12.5%), Penicillium citrinum 1(12.5%) and Rhizopous species 1(12.5%), respectively.

Contamination of UHT milk with moulds and yeasts could give an indication of the neglected hygienic measures applied during processing, packaging and storage. Moreover, they may induce changes that render the product of inferior quality and also constitute a public health hazard among consumers (Lee, 1984).

Fungal contamination of UHT milk indicates errors in storage and defects in packaging processes. Moreover, multiplication of these fungi in UHT milk during storage may induce undesirable flavour, poor appearance and discoloration which lead to economic losses through spoilage of the product (Bullerman, 1981).

Factors that contribute to mycotoxin contamination of food and feed in Africa include environmental, socio-economic and food production. Environmental conditions especially high humidity and temperatures favor fungal proliferation resulting in contamination of food and feed. (Wagacha and Muthomi, 2008).

The data presented in Table (11) revealed that two isolated strains of Aspergillus flavus from pasteurized milk were able to secrete aflatoxn B1, conc. (ppb) with a concentration of 0.07 and 0.92 for both strains respectively. On the other hand, there was only one isolated Aspergillus flavus from UHT milk that able to secrete aflatoxin B1 with a concentration of 0.79 (ppb).

Aflatoxin contamination in milk and dairy products is produced in two ways, either toxins pass to milk with ingestion of feeds contaminated with aflatoxin, or from subsequent contamination of milk and milk product with fungi (Sarimehmetoglu et al. 2003).

The residues of AFM1 remain stable when milk is processed by heat. There is no evidence that cold storage, freezing, heat-treating and concentrating or drying of the contaminated milk changes the level of AFM1(Blanco et al.1988).

The AFM1 level in milk was significantly affected by the geographical region and the country (Ardic et al. 2009). Moreover, differences in the hygiene and storage conditions at the dairies and retail points are other key factors on the variations of results of AFM1 in heat treated milk samples (IARC 1993).

In conclusion the overall hygienic conditions surrounding the production and handling of milk should be monitored.

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