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Epidemiological and Environmental Studies of Dairy Products in the Egyptian Markets

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Abstract

Background: Mankind have been consuming milk and dairy products for tens of thousands of years. Therefore, the aim of this research was to examine pathogenic microbes in dairy products in the Egyptian market and the effect of these microbes as well as the effect of environmental factors. **Methods:** A total of 225 milk product samples were collected in three months period for four kinds of milk products: All samples underwent chemical analysis with Lactoscan in addition to microbiological analysis with the pour plates method, total plates counts, calculation of total and faecal coliforms,Spore forming bacteria,Psychotropic bacteria and thermo bacteria were determined. **Results:** From 225 specimens of dairy products, the average levels of fats, crude proteins, ashes, and total solids were 5.740.78%, 4.710.52%, 0.590.14%, and 14.691.27%, respectively. *Brucella* was detected in 2.22%, *E. coli* in 1.33%, *S.aureus* in 0.44% and *Salmonella* in 0.89% of the total examined samples. Cheese samples contain the highest concentrations of AFM1 with mean values of 3.51 ± 0.34 and 2.79 ± 0.46 µg/Kg in Yogurt and Milk, respectively. **Conclusion:** The microbiological parameters of milk products in this study showed mild levels especially among street vendors' samples. It is recommended that good hygienic practices and regulations, enforce monitoring of milk on regular basis at each channel should be performed.

Keywords: Dairy products, Milk, Egyptian market, epidemiology.

Introduction

Milk and dairy products have long been key components of Egyptian diets, and they continue to play an important and growing role in the diets of the world's growing population, both rural and urban (Jindal et al., 2021).

As a result, milk provides a perfect habitat for a wide range of food-borne bacteria and zoonotic pathogens to thrive. As a result, a wide range of factors and their interactions determine the prevalence of pathogenic and spoilage bacteria in milk and dairy products. These characteristics could include dairy herd health, dairy farm hygiene, milking and presto rage conditions, available storage facilities and technology, farm management techniques, geographic location, and season et al.,2018).

Materials And Methods

This study used a cross-sectional study design to examine pathogenic microbes in dairy products in the Egyptian market and the effect of these microbes as well as the effect of environmental factors. This study was carried out in Cairo, Egypt from 2021 to 2022. The total sample size was calculated as 225 milk samples: 75 bulk milk, 75 yogurt, 50 Fresh feta cheese and 25 processed cheese.

Preparation of samples

Four sections of the samples obtained were aseptic conditions separated for examination. At room temperature (25 °C), each test was run three times.

Laboratory tests for bacterial isolation and identification

The dairy and dairy product testing service examined the sample of milk and other dairy products for their nutritious content and adulteration.

Chemical tests

- Fat determination: calculating fats using the Gerber technique and expressing results as a percentage of milk fats.
- Solid not fat determination: applying the process outlined in Egyptian Standards 155/1974.
- Protein determination: The macro Kjeldahl technique was used to calculate the dairy proteins contents on 10g of milk using the factor N 6.38.
- PH determination: A digitally transportable pH-meter (ST300-B, Ohaus, Parsippany, NJ, USA) adjusted with pH 4 and 7 buffering was used to monitor the pH twice. The standard for raw milk was a pH of 6.6 to 6.8 (de Oliveira, et al., 2013 and Gwandu et al., 2018).
- Ash determination: by heating the dry milk residues at a maximum temperature of 500oC to prevent the chloride ions from volatilizing.
- Calcium determination: as per the procedure outlined in Pearson's Chemical Analysis of Food products Applying Atomics Absorptions Spectroscopy (SCHIMADSU AA-680 flames systems).

Bacteriological Examination

- Total plate count: The samples homogenization was processed into ten-fold dilutions in diluting buffers to produce 10-2, 10-3, and 10-4 dilutions.
- Total coliforms, fecal coliforms and E.coli: Both the totally and faecal coliforms were diluted using several tubes. Lauryl sulphate tryptoses broth (LST) inoculated with 1 ml of prepared 1:10, 1:100, and 1:1000 dilutions was used for the presumptively tests for totally coliforms.
- Staphylococcos aureus: Upon two plates of Baird-Parker agar with egg-yolks tellurite enrichments, one cc of each of the produced diluted sample was placed. 48 hours were spent incubating inverted plates at 37°C.
- Yeasts and Moulds: Every dairy product of the samples was divided into ten grammes, which were then diluted in 90 ml of sterile solutions containing 2% (w/v) sodium citrates and homogenised in a stomacher.

Residue analysis

- Quantitative detection of AFM1: with the use of an enzymes-linked immunosorbents assays (ELISA). Through using Ridascreens AFM1 kits (R-

Biopharm, Derm-Stadt, Germany), AFM1 can be detected (Ghiasian and Maghsood, 2012).

- Quantitative detection of heavy metal residues: Using an atomics absorptions spectrophotometers, the amounts of Fe, Pb, Cu, and Cd were quantified by setting the Cathodes lamps to different wavelength.

Adulteration tests

Experimental procedure: For the examination of all adulterants, samples of market dairy products (1 ml) was put to a MAT kit tubes along with 1 ml of standards reagents.

- Detection of skimmed milk powder (SMP): A sampling of market dairy products (5 ml) was placed in a testing tube, and 10 drops of concentrated nitric acids were then added.
- Detection of dalda ghee: A specimen of commercial milk (3 ml) was placed in testing tubes along with 10 drops of hydrochloric acids and one tea spoon of sugar.
- Extent of extraneous water in market milk: By subtracting the recorded freezing points of commercial milk from the standard freezing points of basis, the amount of extraneous water in the marketing milk products samples were determined (AOAC, 2000).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 20 was used to perform the statistical analysis. Applying descriptive statistical analysis, the average, standard deviation, maximums and minimums values were determined. The Chi-square testing was used to compare various groups with reference to categorical data. The cut-off for significance level was 5% ($P < 0.05$).

Results And Discussion

Physical and chemical analyses:

Data presented in Table (1) ranged, the minimum and maximum values, of the FP milk products samples collected from Egyptian markets (milk, Yogurt, processed cheese and Feta Cheese) was 1.01 to 1.19, 0.34 to - 0.55, 0.26 to 0.55 and 0.28 to 0.53 °C respectively. The differences between the mean values of milk yogurt and processed cheese were significant.

Data of the chemical composition (Table 1) of all samples were studied by variance analysis. A range of 4.15-6.99, 4.25-6.95, 4.12-7.15 and 4.12-7.20% was found for fat, 2.82-4.85, 3.90-5.02, 2.55-4.87 and 2.55-5.02 for protein, 2.66-4.77, 3.30-4.98, 2.96-4.80 and 2.25-4.98 for lactose, 0.42-0.85, 0.42-0.74, 0.47-0.84 and 0.36-0.89 for ash, 11.22-16.45, 12.42-16.44, 11.78-16.41 and 11.39-16.44 for total solids contents in milk, Yogurt, processed cheese and feta cheese respectively. These results indicate that approx. 58% of the samples were not in accordance with Egyptian standards milk products (fat content is not less than 5.50 % and solids not fat (SNF) content is not less than 8.75 %). Our results revealed that the mean concentrations from 225 milk product samples were 5.74±0.78 % for fat, 4.71±0.52 % for crude protein, 0.59±0.14 % for ash and 14.69±1.27% for total solids **(Table 1).**

In a study done by M EL-KHOLY et al., 2018, the fats level varied from 2.00 to 4.00 with an average value of 3.01±0.098 for the analysed raw cow's samples collected, from 2.50 to 3.50 with an average value of 3.10±0.058 for UHT milk, as well as from 27.78 to 76.0 with an average value of 56.99±2.757 for the evaluated heavy creamy specimens.

Table 1: Comparison between the chemical contents of milk product samples in Egypt**:**

Sample	Parameter	FP°C	PH	Fat	Protein	Lactose	Ash	Non-solid	Total solid
Milk (n=75)									
	• **Range**	1.01-1.19	6.1-6.7	4.15-6.99	2.82-4.85	2.66-4.77	0.42-0.85	8.3-11.6	11.22-16.45
	• **Mean± SD**	1.07±0.19	6.61±0.00	5.63±0.90	4.40±0.59	4.61±0.61	0.69 ±0.15	9.07±0.00	14.94±1.16
Yogurt (n=75)									
	• **Range**	0.34-0.55	6.00-6.7	4.25-6.95	3.90-5.02	3.30-4.98	0.42-0.74	8.99-9.3	12.42-16.44
	• **Mean± SD**	0.42 ±0.05	6.65±0.01	6.51±0.61	4.88±0.44	4.29±0.38	0.76±0.11	9.01±0.00	14.26±1.10
Processed cheese (n=25)									
	• **Range**	0.26-0.55	6.002-6.7	4.12-7.15	2.55-4.87	2.96-4.80	0.47-0.84	8.04-8.34	11.78-16.41
	• **Mean± SD**	0.47 ±0.08	6.68±0.00	5.64±0.82	4.91±0.53	4.86±0.56	0.62±0.14	8.24±0.20	14.59±1.43
Feta Cheese (n=50)									
	• **Range**	0.28-0.53	6.3-6.8	4.12-7.20	2.55-5.02	2.25-4.98	0.36-0.89	8.61-8.76	11.39-16.44
	• **Mean± SD**	0.45±0.07	6.69±0.00	5.19±0.79	4.63±0.53	4.85±0.53	0.29±0.14	8.75±0.00	14.99±1.39
Average (n=225)									
	• **Range**	0.28-0.54	6.2-6.9	4.16-7.07	2.95-4.94	2.8-4.88	0.42-0.83	8.33-8.79	11.7-16.44
	• **Mean± SD**	0.45±0.05	6.65±0.01	5.74±0.78	4.71±0.52	4.65±0.52	0.59±0.14	8.74±0.00	14.69±1.27
T-test		38.216*a		3.953*c	50.19*b	1.158			
P-value		<0.001	0.75	<0.001	<0.001	0.162			

n = number of samples. Data are expressed as the Range, mean ± standard deviation (SD), samples were analyzed in duplicate. *Student t-test, statistically significant at P<0.05.

Microbiological analyses:

The total bacteria counts (TBC), coliform, spore forming bacteria and psychrotrophic are presented in Table (2). A difference in the mean of the tested bacterial groups in all milk samples was noticed, which might be due to differences in the sanitation process of the different farmers' markets. Also, 36 % of samples had higher TBC than 6 Log CFU/mL. However, the average of TBC for all samples (5.45±0.51) was in accordance with the Egyptian standards for raw buffalo milk, being 5.70 Log CFU/mL.

The occurrence of the **Coliform group and *E. coli*** in milk products indicates poor hygiene or fecal contamination. Other enteric pathogens may also originate from the external surface during manual milking. Results in Table (2) show that the levels of the coliform group in the three different milk products were higher than the acceptable levels in both the Egyptian standards and the EU specification where the average coliform count was 3.82±0.87 Log CFU/mL. Furthermore, 46 % of the samples had a coliform count of more than 4 Log CFU/mL.

Spore forming bacteria (such as *Bacillus sp*. and *Clostridium sp*.) derive from the farm environment. They can well survive during the pasteurization of milk and grow during refrigerated storage causing milk and its products to spoil and limiting their shelf life. Although there are some regional, seasonal and methodological differences, the range of aerobic spore forming bacteria between the different tested milk products was relatively the same (Table 5).

Psychrotrophic bacteria are defined as those growing at 7 °C. They represent the dominant microflora during the cold storage period and produce heat resistant lipases and proteases causing different dairy products to spoil. Our data in Table (5) show that there was no big variation in the

range of psychrotrophic bacteria between milk (2.16-3.58), Yogurt (2.13-3.39), processed cheese (2.11-3.43) and Feta Cheese (2.16-3.45). The average psychrotrophic bacteria count for all samples was 2.82 Log CFU/mL, indicating the absence of proper standardization of cold storage systems during production and marketing **(Table 2).**

In a study done by Elshaghabee et al., 2017 TBC findings in dairy samples show that hygienic conditions are not sufficient for milk production and commercialization. The TBC for milk in Italy was 5.23 Log CFU/mL Supino et al., 2004 and in China, it was 5.59 log CFU/mL. Han et al., 2007. Nevertheless, in the Alexandria Governorate, the mean TBC of dairy samples was 6.70 Log CFU/mL (Gomaa, et al., 2008) and 7.60 Log CFU/mL in the Menoufia Governorate in Egypt (Ombarak and Elbagory, 2015).

Table 2: Microbiological properties of milk product samples collected from local markets in Egypt**:**

Parameter	Total bacterial count	Coliform	Spore forming bacteria	Psychotropic bacteria	Thermo bacteria
Milk (n=75)					
• **Range**	4.19-6.57	2.11-4.65	2.24-3.41	2.16-3.58	2.25-3.43
• **Mean± SD**	5.42±0.84	3.8±0.88	2.21±0.32	2.89±0.46	2.81±0.36
Yogurt (n=75)					
• **Range**	4.26-6.37	2.18-4.61	2.22-3.44	2.13-3.39	2.25-3.45
• **Mean± SD**	5.28±0.16	3.55±0.95	2.70±0.32	2.72±0.39	2.71±0.33
Processed cheese (n=25)					
• **Range**	4.18-6.53	2.17-4.61	2.22-3.46	2.11-3.43	2.25-3.43
• **Mean± SD**	5.51±0.81	3.94±0.85	2.88±0.34	2.88±0.49	2.84±0.39
Feta Cheese (n=50)					
• **Range**	4.19-6.51	2.11-4.62	2.23-3.46	2.16-3.45	2.25-3.43
• **Mean± SD**	5.59±0.85	3.98±0.81	2.88±0.34	2.81±0.46	2.78±0.36
Average (n=225)					
• **Range**	4.21-6.49	2.19-4.62	2.23-3.44	2.14-3.46	2.14-3.51
• **Mean± SD**	5.45±0.51	3.82±0.87	2.67±0.33	2.82±0.45	2.88±0.40
T-test	26.216*a	6.953*c	2.19	1.69	
P-value	<0.001	<0.001	0.44	0.187	

n = number of samples. Data are expressed as the Range, mean ± standard deviation (SD), samples were analyzed in duplicate. *Student t-test, statistically significant at P<0.05.

Cheese samples contain the highest concentrations of AFM1 with mean values of 3.51 ± 0.34 and 2.79 ± 0.46 µg/Kg in Yogurt and Milk, respectively. These values may be due to the affinity of AFM1 for milk protein, mainly casein, therefore AFM1 is highly concentrated in Yogurt than in milk **(Table 3).**

In the study by Elshaghabee et al., 2017, AFM 1 concentrations are substantially (P= 0.05) greater in central Qualubya (143.58 ng/L) than those are in downtown Cairo (57.26 ng/L) and Giza (29.68 ng/L).

Table (3): Occurrence of AFM1 in dairy product samples collected from local markets in Egypt

Sample	Examined	Positive	Yeast and Mold	Min-max (µg /Kg)	Mean ±SD (µg

	samples	samples N (%)	(CPU/ml)		/Kg)
Milk					
	75	5 (6.7%)	<10	0.97 – 5.72	2.79 ± 0.46
Yogurt					
	75	3 (4%)	<10	1.06 – 3.67	3.51 ± 0.34
Processed cheese					
	25	2 (8%)	<10	0.37 – 0.85	0.45 ± 0.06
Feta Cheese					
	50	3 (6%)	<10	0.39 – 0.92	0.47 ± 0.08

n = number of samples. Data are expressed as the Range, mean ± standard deviation (SD), samples were analyzed in duplicate.

The concentrations of heavy metals in dairy products are presented in table (4) where the concentrations of Fe varied from 0.32 to 7.70 ppm. The highest mean value was found in milk samples, while the lowest one was found in Feta Cheese. The high concentration of Fe in milk and dairy products represents a problem in dairy technology due to its catalytic effect on lipid oxidation and its bounding proteins and membrane lipoproteins of the milk fat globule with the development of unpleasant smell **(Table 4).**

The ranges of irons levels in the specimens of milk, kareish cheese, butter, and rice pudding were 2.9619–45.6198, 1.7633–14.7388, 5.0693–13.14, and 1.3208–3.438 ppm, correspondingly, with average scores of 8.994±1.87, 3.93±0.67, 6.69±0.437, and 2.04±0.17 ppm (Meshref et al., 2014).

Table (4):- Mean levels of heavy metals in the examined dairy product samples:

Metals		Milk	Yogurt	Processed cheese	Feta Cheese
Fe (ppm)	Min.	6.32	3.14	1.23	1.25
	Max	7.70	5.23	1.55	1.73
	Mean ±SE	7.07± 0.14	4.23± 0.36	1.36± 0.065	1.32 ± 0.077
Pb (ppm)	Min.	0.81	0.77	0.36	0.32
	Max	1.35	1.47	0.58	0.51
	Mean ±SE	0.96± 0.07	0.95± 0.14	0.55± 0.04	0.45 ± 0.07
Cu (ppm)	Min.	1.15	0.91	0.74	0.72
	Max	2.14	1.73	1.28	1.36
	Mean ±SE	1.66± 0.12	1.24± 0.23	0.81± 0.06	0.79 ± 0.08
Cd (ppm)	Min.	0.23	0.18	0.15	0.19
	Max	0.32	0.32	0.37	0.32
	Mean ±SE	0.27± 0.04	0.23± 0.02	0.18± 0.01	0.20 ± 0.009

Table 5 indicates the detection of adulterants in all diary milk samples. It was noticed that cane-sugar was detected in 16 samples. Urea was detected in 32 samples. Ammonium sulphates were detected in 31, Glucose in 10, and Sodium chloride in 19 samples, **(Table 5).**

Table (5): Detection of adulterants in all diary milk samples (n=225):

Adulterants	Test	+Ve Sample of all diary product samples (n=225)
Cane- sugar	Resorcinol test	16
Urea	p-DMAB test	32
Ammonium sulphates	Nessler's reagent test	31
Glucose	Modified Barfoed reagent & Phosphhomolybdic acid reagent	10
Skimmed Milk powder	Conc.HNO3 reagent	-
Sodium chloride	Silver nitrate + potassium chromates reagent	19

Conclusion

In this investigation, the microbiological characteristics of dairy products revealed low concentrations, particularly in samples from street vendors. It could be concluded that the non-heat treated milk and its products have risky role in transmission of pathogenic bacteria such as *E. coli, B. cereus* and *S. aureus* to humans where the milk become contaminated with foodborne pathogens either through direct or indirect contact with contamination reservoirs in the farm or from the mastitic udder. It is recommended that good hygienic practices and regulations, enforce monitoring of milk on regular basis at each channel and to identify sources of contamination in the dairy supply chain, novel pathogens and their food vehicles, and dairy supply chain gaps that endanger food safety.

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