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ORIGINAL ARTICLE

Microbiological Study of Some Cheese and Milk Powder Samples

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Key words:

Soft cheese, Processed cheese, Hard cheese, Milk powder, E.coli, Salmonella spp., Moulds, Yeasts, Total aerobic count, Coliform count

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ABSTRACT

***Background**: Milk is a nutritious food for human, but it is also a suitable medium for growth of microorganisms and can spoil easily. **Objective**: The aim of this study was to establish the microbidogical quality of cheese and powdered milk in order to prevent potential risks of dairy products for consumer health and to ensure its quality. **Methodology**: A total of seventy five samples of different cheese types (Soft Cheese, Processed cheese and Hard cheese, 25 each) and fifty samples of milk powder were collected from different supermarkets in Mansoura City, Dakahlia Governorate. All samples were evaluated microbiologically for the quality of this products through determination of total bacterial count, coliforms count and Moulds and Yeasts count. Isolation and identification of E.Coli, Staphylococcus aureus, Salmonella spp., moulds and yeasts were also carried out. **Results**: In Cheese Samples, the mean value of total aerobic count were $1.2\times10^4\pm2.1\times10^3$, $2.2\times10^3\pm3.3\times10^2$, $1.1\times10^4\pm6.2\times10^3$ cfu/gm for Soft, Processed and Hard Cheese, respectively. The mean value of Coliform count were $2.3\times10^3\pm0.21\times10^3$, $1.2\times10^2\pm0.33\times10^2$, $3.1x10^2\pm0.56\times10^2$ cfu/gm for Soft, Processed and Hard Cheese, respectively. The mean value of mould and yeast count were $3.2\times10^3\pm0.12\times10^3$. $1.1\times10^2\pm0.13\times10^3$, $1.6\times10^2\pm0.75\times10^2$ cfu/gm for Soft, Processed and Hard cheese, respectively. Salmonella spp. appeared only in 20% of Soft cheese samples, while it was absent in Processed and Hard cheese samples. Staphylococcus aureus were absent in Hard cheese, while it was detected in 60% and 32% of Soft and Processed cheese, respectively. The mean value of E.coli were $3.2\times10^3\pm0.12\times10^3$, $1.1\times10^3\pm0.13\times10^3$ cfu/gm for Soft and Processed cheese, respectively. While it was not detected in Hard Cheese samples. In milk powder samples, the mean value of total aerobic count was $2.1\times10^3\pm0.12\times10^3$ cfu/g. Total coliforms count of examined samples was 12-55 cfu/gm in 4(8%) samples, while coliforms were not found in 46 (92%) of milk powder samples. The mean value of moulds and yeasts count was $1.3\times10^2\pm0.67\times10^2$, which was insignificant. Salmonella spp. and E.coli were not detected in all milk powder samples, while Staphylococcus aureus was found in 18% of the examined samples. **Conclusions**: From the results of this study, it could conclude that the unhygienic conditions prevailing during handling, processing, distribution or sale should be reviewed and controlled by respective authority in order to prevent contamination of cheese and powdered milk for the safety of consumers*

INTRODUCTION

Milk is a nutritious food for human, but it is also a suitable medium for growth of microorganisms and can spoil easily. Therefore, in order to increase its resistance and to obtain different nutritious dairy products, milk is processed into different products such as butter cheese, dried milk, ice cream and condensed milk[1].

Cheese making practice is an ancient milk preservation technique. It was traditionally made on small scale caning milk in animal skin sacks, stomachs or bladders. Over centuries, the cheese making technique has been modified and finally evolved into a large scale commercial process in which scientific principles are applied[2].

Dried milk powder must exhibit high quality in sensory, nutritional and microbiological attributes at the time of purchase if quality is to be maintained during long-term storage[3]. In many developing countries e.g. Egypt, the shortage in milk supply requires increasing use of milk powder. The consumer reconstitutes the milk powder whether add it to hot beverages, cheese, yoghurt, bakery products and infant formula to make their nutritional content the same as the existing food[4].

The microbiological quality of milk and dairy products is influenced by the initial flora of raw milk, the processing conditions and post-heat treatment contamination. Therefore, poor sanitary practices in local cheese processing and distribution results in public health hazard due to the presence of pathogenic bacteria, mold and yeast. Undesirable microbes that can

cause spoilage of dairy products include Gram-negative psychrotrophs, coliforms, lactic acid bacteria, yeasts and molds. In addition, various bacteria of public health concern such as Salmonella spp., Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica, pathogenic strains of Escherichia coli and enterotoxigenic strains of Staphylococcus aureus may also be found in milk and dairy products[5].

Microorganisms, including bacteria, yeast and molds, are present in cheese throughout ripening and have effects on maturation process. They contribute either directly through their metabolic activity or indirectly through the release of enzymes into the cheese matrix through autolysis[6].

Dairy products are characterized by reduced shelf life because they are excellent growth medium for a wide range of microorganisms. Therefore, the aim of this work is to establish the microbiological quality of cheese and powered milk in order to prevent potential risks of dairy products for consumer health and to ensure its quality.

METHODOLOGY

This work was carried out in the Microbiology and Immunology Department, Faculty of Medicine, Mansoura University during the period form June 2016 to October 2016.

Samples:

One hundred and twenty five random samples of Damietta (Soft) cheese, Processed cheese, Hard cheese (25 of each) and 50 samples of milk powder were randomly purchased from different markets in Mansoura city. All samples were tight and free from any damages. The collected samples were transferred directly to the laboratory in refrigerant at 4ºC where they were examined microbiologically without delay.

Preparation of samples:

Cheese Samples:

10 g of cheese was taken and homogenized in sterile 90 ml of 2% sodium citrate at 45ºC. Serial dilutions in sterile 0.1% peptone water were prepared for bacteriological analysis[7].

Milk Powder Samples:

10 g of milk powder was reconstituted in 90 ml of water of excellent microbial quality at 25ºC. The powder was mixed thoroughly in the water and then held in a covered glass container in the dark for an hour at 20ºC. The reconstituted milk was examined for colour, flavor, odor and appearance[8].

Microbiological analysis of cheese and powdered milk samples:

Enumeration of microorganisms:

Serial dilutions of samples were made up to 10^7 in sterile normal saline and tested for total viable counts at 30ºC. Most probable number (MPN) was used for determining coliform counts and molds and yeasts counts at 25ºC, following the methods given in Standard Method for the Examination of Dairy products[9].

Isolation of E. Coli and Staphylococcus aureus

Twenty five ml of prepared sample was added to 225 ml of buffered peptone water and incubated at 37ºC overnight. For isolation of E.coli, the enriched sample was cultured on selective medium Eosin Methylene Blue (EMB) agar and incubated at 37ºC for 24 hours. Morphologically typical colonies (at least 4/plate) producing metallic sheen were taken into nutrient agar for further identification. For isolation of S. aureus, enriched samples were streaked on Baird Parker Agar (BPA) and incubated at 37ºC for 24-48 hours. Appearance of jet black colonies surrounded by white halo were considered to be presumptive S. aureus[10].

Identification of E. Coli and Staphylococcus aureus:

The shape and type of Gran reaction are microscopically studied using 18 hour culture from agar plate. Identification of isolates obtained in pure culture was based on Gram staining, biochemical characteristics and growth pattern on selective and differential media according to the procedures recommended in the Bergey's Manual of Determinative Bacteriology[11].

Isolation and Identification of Salmonella Spp.:

Presumptive colonies of Salmonella spp. were transferred from Desoxycholat Citrate agar to nutrient agar many times until purified. All strains were tested by Gram stain and oxidase activity [12]. The full identification was performed by using API 20E kit (BioMerieux, Marcy l' Etoile, France).

Isolation and Identification of Mould and Yeast:

This was accomplished by culturing the prepared samples on Sabouraud's Dextrose Agar and the suspected colonies were examined microscopically[13].

Statistical Analysis:

Mean values, standard deviations, maximum and minimum values were calculated for all the determined parameters using SPSS (version 13; SPSS Institute Inc., Chicago, IL, USA).

RESULTS

All examined cheese samples of soft (Damietta) cheese, Processed cheese and Hard cheese contain total aerobic bacterial count of $2.3\times10^2 - 3.0\times10^6$, $1.2\times10^2 - 2.6\times'10^5$, $3.2\times10^2 - 1.2\times10^5$ with the mean value of $1.2\times10^4 \pm 2.1\times10^3$, $2.2\times10^3 \pm 3.3\times 10^2$ and $1.1\times10^4 \pm 6.2\times 10^3$ cfu/g, respectively. Table (1).

Table 1: Statistical analytical results of total aerobic count/gm of examined commercial cheese samples.

Products	*No. of examined samples*	*Positive Samples*		*Total bacterial count (TBC/g)*		
		No.	**%**	**Min**	**Max**	**Mean ± S.E.**
Soft cheese	25	25	100	2.3×10^2	3×10^6	$1.2 \times 10^4 \pm 2.1 \times 10^3$
Processed cheese	25	25	100	1.2×10^2	2.6×10^5	$2.2 \times 10^3 \pm 3.3 \times 10^2$
Hard cheese	25	25	100	3.2×10^2	1.2×10^5	$1.1 \times 10^4 \pm 6.2 \times 10^3$

Coliforms count of examined Soft cheese, Processed cheese and Hard cheese samples revealed that 80%, 60 and 68%, respectively, of these samples contain a range of $1.2\times10^2 – 2.0 \times 10^4$, $0.4 \times 10^2 – 3.2 \times 10^3$ and $0.2 \times10^2 – 1.2 \times 10^3$ with a mean value of $2.3 \times 10^3 \pm 0.21 \times10^3$, $1.2\times 10^2 \pm 0.33 \times 10^2$ and $3.1 \times 10^2 \pm 0.56 \times 10^2$ cfu/gm, respectively. Table (2).

Table 2: Statistical analytical results of coliforms count/gm of examined commercial cheese samples.

Products	*No. of examined samples*	*Positive Samples*		*Coliforms count /gm*		
		No.	**%**	**Min**	**Max**	**Mean ± S.E.**
Soft cheese	25	20	80	1.2×10^2	2×10^4	$2.3 \times 10^3 \pm 0.21 \times 10^3$
Processed cheese	25	15	60	0.4×10^2	3.2×10^3	$1.2 \times 10^2 \pm 0.33 \times 10^2$
Hard cheese	25	17	68	0.2×10^2	1.2×10^3	$3.1 \times 10^2 \pm 0.56 \times 10^2$

The results of this work showed that the examined Hard cheese samples were free from true fecal type E. coli, while 48% and 32% of the examined Soft cheese and Processed cheese samples contain true fecal type E. coli ranged from $2.1 \times 10^2 – 1.2 \times 10^4$ and $0.4 \times 10^2 – 2.8 \times10^3$ cfu/gm, respectively. Table (3) .

Table 3. Statistical analytical results of true fecal type E. coli of examined commercial cheese samples.

Products	*No. of examined samples*	*Positive Samples*		*true fecal type E. coli/gm*		
		No.	**%**	**Min**	**Max**	**Mean ± S.E.**
Soft cheese	25	12	48	2.1×10^2	1.2×10^4	$3.2 \times 10^3 \pm 0.12 \times 10^3$
Processed cheese	25	8	32	0.4×10^2	2.8×10^3	$1.1 \times 10^3 \pm 0.13 \times 10^3$
Hard cheese	25	0	0	0	0	0

Regarding yeast and mold counts, the examined Soft cheese, Processed cheese, and Hard cheese samples showed a range of $0.33 \times 10^2 – 2.0 \times 10^3$, $0.08 \times 10^2 – 1.3 \times10^2$ and 0.13×10^2 - 6.1×10^2, respectively. Table (4) .

Table 4: Statistical analytical results of as Yeast & mould count/gm of examined commercial cheese samples.

Products	*No. of examined samples*	*Positive Samples*		*Yeast & mould count/gm*		
		No.	**%**	**Min**	**Max**	**Mean ± S.E.**
Soft cheese	25	25	100	0.33×10^2	2×10^3	$3.2 \times 10^3 \pm 0.12 \times 10^3$
Processed cheese	25	12	48	0.08×10^2	1.3×10^2	$1.1 \times 10^3 \pm 0.13 \times 10^3$
Hard cheese	25	4	16	0.13×10^2	6.1×10^2	$1.6 \times 10^2 \pm 0.75 \times 10^2$

On the other hand, Salmonella spp. was not detected in all Processed and Hard cheese samples, while 20% (5 samples) of soft cheese samples gave positive results. Moreover, Staphylococcus aureus was not found in all Hard cheese samples, while 60% and 32% of Soft cheese and Processed cheese samples showed positive results, Table (5).

Table 5: Detection of some bacterial pathogens in examined commercial cheese samples.

Products	*No. of examined samples*	*Salmonella spp.*		*Staph. aureus*		*E.coli*	
		No. of positive samples	**%**	**No. of positive samples**	**%**	**No. of positive samples**	**%**
Soft cheese	25	5	20	15	60	12	48
Processed cheese	25	0	0	8	32	8	32
Hard cheese	25	0	0	0	0	0	0

By estimating the total viable bacterial count of milk powder samples, it ranged from 0.45×10^2 to 5.11×10^3 with a mean value of $2.1 \times 10^3 \pm 0.12 \times 10^3$ cfu/gm. Table (6)

Table 6: Statistical analytical results of total bacterial count/gm of examined milk powder samples.

Products	*No. of examined samples*	*Positive Samples*		*Total bacterial count (TBC/g)*		
		No.	**%**	**Min**	**Max**	**Mean ± S.E.**
Milk Powder	50	50	100	0.45×10^2	5.11×10^3	$2.1 \times 10^3 \pm 0.12 \times 10^3$

On the other hands, total coliforms count in milk powder specimens gave 12-55 cfu/gm in four (8%) specimens only, while coliforms were
not found in 46 (92%) of milk powder specimens. Table (7)

Table 7: Coliforms count and True fecal type E.coli of examined milk powder samples.

Products	*No. of examined samples*	*Coliform count/gm*		*True fecal type E.coli/gm*	
		No. of Positive samples	**%**	**No. of Positive samples**	**%**
Milk Powder	50	4 (12 – 55) Range count/gm	8	0	0

Regarding yeast and mold counts of milk powder, they were detected in all examined milk powder specimens giving a range from 0.8×10^2 to 2×10^2 with a mean value of $1.3\times10^2 \pm 0.67\times10^2$. Table (8).

Table 8: Statistical analytical results of Yeast & Mould counts of examined milk powder samples.

Products	*No. of examined samples*	*Positive samples*		*Yeast & Mould counts/gm*		
		No.	**%**	**Min**	**Max**	**Mean ± S.E.**
Milk Powder	50	50	100	0.08×10^2	2×10^2	$1.3 \times 10^2 \pm 0.67 \times 10^2$

By studying the bacterial pathogens in examined milk powder specimens, Staphylococcus aureus was found in 18% of specimens, while Salmonella spp. and E.coli were not detected. Table (9).

Table 9: Detection of some bacterial pathogens in examined milk powder samples.

Products	*No. of examined samples*	*Salmonella spp.*		*Staph. aureus*		*E.coli*	
		No. of Positive samples	**%**	**No. of Positive samples**	**%**	**No. of Positive samples**	**%**
Milk Powder	50	0	0	9	18	0	0

In a trial to compare between the results obtained from the microbiological (bacteria, yeasts and molds) examination of the studied cheese and milk powder specimens with Egyptian Standards (ES), these findings were arranged in the following tables: Table 10, 11, 12.

Table 10: Comparison between the results obtained from microbiological examination for examined milk powder samples and Egyptian Standards ES/ 1648/2005.

Parameter	*Egyptian Standards (ES)*	*Milk powder samples comply with ES*	
		No.	**%**
Total bacterial count	10^4/g	50	100
S. aureus	Nil	41	82
E. coli	Nil	50	100
Salmonella spp.	Nil	50	100
Yeast & moulds count	10/g	9	18

Table 11: Comparison between the results obtained from microbiological examination of examined commercial cheese samples and Egyptian Standards ES/ 1007-1/2005.

Parameter	*Egyptian Standards (ES)*	*Soft cheese comply with ES*		*Processed cheese comply with ES*		*Hard cheese comply with ES*	
		No.	**%**	**No.**	**%**	**No.**	**%**
Coliform count	≤ 10/g	5	20	10	40	8	32
S.aureus count	Nil	10	40	17	68	25	100
Salmonella	Nil	20	80	25	100	25	100
E.coli	Nil	13	52	17	68	25	100

Table 12: Comparison between the results obtained from yeast and mould counts of examined commercial cheese samples and Egyptian Standards ES/ 1007-1/2005.

Product	*ES*	*Comply with ES*	
		No.	**%**
Soft cheese	400/g	12	48
Hard cheese	100/g	9	36
Processed cheese	10/g	6	24

DISCUSSION

Microbial contamination, causing about one-fourth of the world's food supply loss, has become an enormous economic and ethical problem worldwide [14]. Dairy products are an excellent growth medium for a wide range of microorganisms and thus, display a reduced shelf life[15]. The microbiological quality of dairy products is influenced by the initial flora of raw milk, the processing conditions and post-heat treatments. Spoilage bacteria and various bacteria of public health concern can be found in these products and their concentrations should be kept as low as possible[16].

In this work, all cheese specimens of Soft cheese, Processed cheese and Hard cheese gave total aerobic bacterial count of $2.3\times10^2 - 3\times10^6$, $1.2\times10^2 - 2.6\times10^5$ and 3.2×10^2–1.2×10^5 cfu/gm, respectively.

Higher bacterial count of 10^{11}–10^{12} cfu/gm was found by other workers in Soft cheese [17]. Total bacterial count ranged from 10^7 to 10^9 was reported by another author in Kariesh cheese[18], while other investigators estimated total bacterial count up to 10^6 in Processed cheese, Kariesh cheese and Soft cheese[19,20], which indicates bad microbiological quality.

The results of this research revealed that 80%, 60% and 68% of the examined Soft cheese, Processed cheese and Hard cheese specimens, respectively, contain coliforms count of 1.2×10^2–2.0×10^4, 0.4×10^2–3.2×10^3 and $0.2\times!0^2$–1.2×10^3 cfu/g, respectively.

Egyptian Standard ES/1007-1/2005 implies that cheeses must not contain more than 10 coliforms/g, and so about 80%, 60% and 68% of the examined Soft cheese, Processed cheese and Hard cheese specimens, respectively, failed to comply with Egyptian Standard (ES).

Lower values were estimated by many authors who found that coliforms were not detected in 95% of examined samples, while only 5% had coliforms of 3-10/g[21]. Other workers reported that all samples were free from coliforms[22], while others estimated coliforms count of 102 cfu/g, which is considerably low[23].

The findings of this work pointed out that all Hard cheese specimens were from true fecal type E.coli, while 48% and 32% of examined Soft cheese and Processed cheese specimens, respectively contain true fecal type E.coli.

Egyptian Standard ES/1007-1/2005 implies that cheeses must be free from E.coli, and so 48% and 32% of the examined Soft cheese and Processed cheese samples, respectively, failed to comply with Egyptian Standard, while all studied Hard cheese specimens meet ES.

Many workers could not find true fecal type E.coli in Processed cheese[24], Soft cheese and Fayoumi cheese[26]. On the other hands, higher count was recorded by another author[27]. Certain food-borne yeasts and molds may be harmful because of their ability to elicit allergic reactions[28]. The findings in the present work showed that 100%, 48% and 16% of the studied Soft cheese, Processed cheese and Hard cheese specimens, respectively, contain yeast and mold. In other words, this work revealed that 52%, 64% and 76% of the examined Soft cheese, Processed cheese and Hard cheese samples failed to comply with Egyptian Standard ES/1007-1/2005 for yeast and mold count.

In the present work, the results of microbiological analysis for detection of bacterial pathogens in the studied cheese specimens pointed out that Salmonella spp. was not found in all Processed and Hard cheese specimens, while 20% of Soft cheese specimens gave positive results.

On the other hands, Staphylococcus aureus was not detected in all Hard cheese samples, while 60% and 32% of Soft cheese and Processed cheese samples,

respectively, showed positive results. So, 100% of Processed and Hard cheese samples comply with Egyptian Standard ES/1007-1/2005 for absence of Salmonella spp., while 20% of Soft cheese samples failed to meet ES. Again, 100% of Hard cheese samples, in this study, comply with Egyptian Standard ES/1007-1/2005 for absence of Staphylococcus aureus, while 60% and 32% of Soft cheese and Processed cheese samples, respectively, failed to comply with ES.

The findings of this study were in agreement with that of other researchers who could not detect Salmonella spp. in any of the examined samples[29]. Moreover, another worker failed to detect salmonella spp. neither in Mozzarella cheese samples not in Cheddar cheese samples[30]. On the reverse, other investigators detected Salmonella spp. in 23.3% of examined cheese samples[31]. On the other hands, many workers could not detect Staphylococcus aureus in all studied cheese specimens[32], while other authors confirmed contamination by Staphylococcus aureus above the accepted level[33].

Production of milk powder is a simple process carried out on a large scale. It involves the removal of water under stringent hygienic conditions while retaining the desirable natural properties of the milk; color, flavor, solubility and nutritional value. Steps of milk powder production involve: collection of raw milk, pasteurization, centrifugation, preheating evaporation, spray drying, packaging and storage[34].

In the present study, the total viable bacterial count of the examined milk powder specimens ranged from 0.45×10^2 to 5.11×10^3 cfu/g. These results clearly show that all milk powder specimens contain less than the upper limit (10.000/g) required by the Egyptian Standard, 2005. According to the Codex Alimentarius Commission[35], the dried milk powder should contain less than 5.0×10^4 cfu/g, and according to the specification given by ICMSF[36], it should be less than 3.0×10^4 cfu/g. In this study, aerobic plate counts for most of the samples were within higher aerobic plate counts ranged from 1.4×10^2 to 4.4×10^4 cfu/gm which was reported by other workers [37].

Milk powder is generally considered a product of good microbiological quality, considering that it made from good quality milk and containing low microbial count and the moisture content is kept low[38].

In the current research, total coliforms count of four (8%) milk powder samples was 12-55 cfu/g, while coliforms were not found in 46 (92%) of samples and this complies with the Egyptian Standards. Coliform organisms in dried milk powder might die out during processing and storage and its presence indicates poor post –processing sanitation since their presence is often used as a post-processing hygienic indicator.

It is known that coliforms are destroyed by pasteurization and by successive heat treatment during processing. Therefore, the presence of these bacteria in dried milk powder may be attributed to contamination after heating caused improper clearing and sterilization of manufacturing utensils. However, even if milk gets contaminated with low level of coliforms as do some serotypes of E.coli, they would be able to proliferate and produce millions of cells during holding at warmer temperatures which may cause some health hazards such as diarrhoeal diseases [39].

In the present work, the counts of yeasts and molds of dried milk samples were very little in all samples and can be insignificant. Some species of moulds can produce mycotoxins which are implicated in human cases of food poisoning and neoplastic diseases including hepatoma, leukemia and other cancers. On the other hand, some species of yeasts constitue a public health hazards such as gastrointestinal disturbances[40].

In this study, the microbiological analysis for detection of some bacterial pathogens revealed presence of Staphylococcus aureus in 18% of examined milk powder specimens, which indicates post-processing contamination of the milk. On the other hands, Salmonella spp. and E.coli were not detected from any of the samples tested. Furthermore, 82%, 100% and 100% of examined milk powder specimens complied with Egyptian Standards, 2005 regarding these isolates, respectively.

Salmonella spp., in this work, was not isolated from any of the tested milk powder specimens and this agreed with other authors[37]. On the reverse, some investigators detected Salmonella spp. in 20% of examined milk powder samples and linked this finding to the high total viable bacterial and coliforms counts of the studied specimens[41].

Staphylococcus aureus has been linked to gastroenteritis by producing enterotoxins, boils, abscesses, pneumonia and meningitis in debilitated persons. The presence of Staphylococcus aureus and E.coli will render milk unfit for human consumption, since sufficient number of the organisms will cause infection and intoxication. Multiplication of these bacteria however, depends upon environmental factors such as temperature, relative humidity and duration of storage and food factors like moisture content, additives and associated microflora[42].

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