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Microbiological Quality of Commercial Raw Milk, Domiati Cheese and Kareish Cheese

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ABSTRACT

The purpose of this research was to assess the microbiological quality of commercially raw milk, Domiati cheese and Kareish cheese. The products surveyed included 50 samples of raw milk, 50 samples of Domiati cheese and 30 samples of Kareish cheese were collected from Cairo governorate markets and microbiologically examined. Coliform group , *Escherichia coli, Escherichia coli* O157 and *Salmonella* spp. were implicated in 100, 80 ,40 ,50% of raw milk, 80 ,20,4,4% of Domiati cheese and 100 , 33, 6.7,6.7 % of Kareish cheese samples respectively. The average counts of *Staphylococcus aureus*, and yeasts & molds were 5.3, 3.5 log cfu / ml of raw milk samples and 4.4, 3.3 log cfu / gm of Domiati cheese and 5.3, 5.5 log cfu / gm of Kareish cheese respectively. In conclusion, it was observed that the hygienic quality of white cheeses sold in dairy shops in Cairo city was low and does not have enough assurance in terms of public health. These results emphasize the need for applying more strict hygienic practices especially in traditional cheese processing to minimize microbial contamination.

Key words: Raw milk, Domiati cheese, Kareish cheese, Microbiological Quality, Pathogenic bacteria

Introduction

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. 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 (Rajagopal *et al*., 2005). Domiati cheese is the most type of pickled white soft cheese in Egypt. It is consumed either fresh or after pickling for few months. The microbial quality and safety of Domiati cheese is the major area of concern for producers and consumers .Also, Kareish cheese is a soft cheese commonly made and consumed in Egypt. This cheese is an excellent source of protein, amino acids, calcium, phosphorus, vitamins and many micronutrients. Environmental conditions prevailing during storage, combined with the composition of the cheese often create possibilities for extensive development of mould on cheese surface, which reduces considerably its quality (Reps *et al*., 2002). The microbiological quality of cheese is influenced by quipments and environmental hygiene during manufacturing, packaging and handling (Robinson and Tamime, 2002).

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. For this reason, increased emphasis should be placed on the microbiological examination of milk and dairy foods (Oliver *et al.,* 2009). Microbiological investigations for the different varieties of Egyptian white soft cheese have been carried out either to evaluate their qualities, hinder or minimize microbial spoilage and to determine the cheese safety as free from foodborne microorganisms. Abou- Dawood *et al.,* (2005) found that microbiologically, 10 % of the Kareish cheese samples were positive for *Salmonella.* All cheese samples had higher molds & yeast counts than that allowed by the legal standards. As for Kariesh cheese they found that the samples had higher moisture content, higher coliforms and molds & yeast counts than the standard requirements. Also, Hassan and Elmalt (2008) reported that *E .coli* were recovered from 38 (76%) of raw milk and 11 (47.8%) of Kareish cheese samples. El Sayed *et al.,* (2011) found that coliform group and *Escherichia coli* as fecal indicator contamination were implicated in 50 and 24 % of the retailed white soft cheese samples, respectively. The pathogenic *E.coli* O157H7 has been isolated from 19% of the total samples. Also, *Salmonella* spp, has been only isolated from Domiati and Tallaga cheese varieties in 3 and 7 % of the samples, respectively. Soliman *et al*., (2004), summarized the reasons for contamination of dairy products as follows, the used milk is either raw or pasteurized. Cheeses are made either in large planning that is well equipped or in small planning or in farmers' home or in unlicensed factories. The last three places specially the unlicensed one is a disaster. Manufacturing cheese in these places make them more labile to contamination and any fault during manufacture may lead to series hazards. Microorganisms may gain access to cheese during process; handling and distribution

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since milk provide a high nutritive, favorable media for the growth and multiplication of such organisms. Many food poisoning outbreaks may be due to using milk from diseased animals with infection of bacterial origin or manufacturing in contaminated places or from the workers themselves. Ingestion of certain microorganism can be detrimental to human health. Heikal *et al.,* (2014), confirmed previous reasons. They reported that fresh soft cheeses are made by using only traditional methods in the different geographical locations in Egypt. The traditional method of manufacture involves renneting, curd formation and preparation for markets. This cheese is made from raw milk (usually heat-treated) without starter cultures. Microbial contamination of cheese may originate from various sources. Such sources might be during cheese production (Temelli *et al*., 2006), storage (Brito *et al*., 2008) or from humans contamination (Callon *et al*., 2008).

Therefore, the aim of this study was to monitor the microbiological quality of milk and cheese sold in Cairo, Egypt.

Material and Methods

Samples collection

A total of 130 samples of the Egyptian raw milk and soft cheese (50 samples of raw milk, 50 samples of Domiati cheese and 30 samples of kareish cheese) were collected from Cairo governorate markets and microbiologically examined.

Microbiological examination

Total bacterial bacterial count

The aerobic colony count (TBC) was carried out as the conventional method, FDA, (2002*)* using plate count agar (Oxoid).

Determination of coliforms and Escherichia coli

Coliform group was determined using solid medium method onto plates of violet red bile agar (Difco) according to the method reported by, FDA, (2002). Positive tubes were streaked onto MacConkey agar (Merck, Germany) according to, APHA, (1976). Suspected red colonies were tested for IMVIC test for typical *E.coli.* Enteropathogenic & enterotoxigenic *E.coli* identification within the (+) IMVIC test isolates were examined using the serological reactions and indicators.

Detection of Escherichia coli O157: H7

Samples dilutions were spread onto plates of medium Sorbitol Mac Conkey agar (Oxoid, England). After 18-24h at 35º C incubation, sorbitol negative colonies (pale - colored, typical E. coli 0157: H7) were serologically tested, as outlined by, FAD, (2002).

Enumeration of Staphylococcus aureus:Enumeration of *Staph. aureus* in the samples was carried out by, APHA, (1976) and FDA, (2002).
Enumeration of *Bacillus cereus*: *Bacillus cereus* was determined by the surface plating technique onto the manitol egg yolk - polymyxin ager, MYPA, Oxoid,(2005).
Detection of Salmonella was carried out by enrichment using selenite cystein broth (Oxoid). Plates of *Salmonella & Shigella* ager (SS) were streaked.
Molds and yeasts counts: Enumeration and counts of yeasts and molds were carried out in the samples using the media of acidified potato dextrose agar (Oxoid).

Results and Discussion

Microbiological evaluation of marketed raw milk

Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. 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. For this reason, increased emphasis should be placed on the microbiological examination of milk and dairy foods. Microbiological analyses are critical for the assessment of quality and safety, conformation with standards and specifications, and regulatory compliance.

Table 1: Microbiological properties of 50 raw milk samples

Type of Microorganism	Positive samples		Counts (log cfu./ ml)		
	No	%	Min.	Max.	Av.
Total bacterial count	50	100	6.2	8.4	7.8
Staph.aureus	50	100	4.7	5.7	5.3
Coliform group	50	100	4.8	6.3	5.8
E.coli	40	80	--	--	--
E.coli O157	20	40	--	--	--
Salmonella	25	50	--	--	--
Bacillus cereus	ND	ND	--	--	--
Yeasts & molds	50	100	2.8	4.3	3.5

Min. Minimum Max. Maximum Av. Average ND Not Detected

Results given in Table (1) revealed that the TBC of raw milk ranged between 6.2 and 8.4 log cfu/ml, with an average 7.8 log cfu/ml. These results are higher than those found by Godic Torkar and Golceger (2008) and Neelu and Shobha (2014), but less than that reported by Uddin *et al.,* (2011). The Table also show that the numbers of coliform bacteria were higher compared with other microbial groups. Data also show that the numbers of coliform bacteria ranged between 4.8 to 6.3 log cfu/ml with an average of 5.8 log cfu/ml which is much higher than those found by Godic Torkar and Golceger (2008) and Neelu and Shobha (2014) but less than that reported by Uddin *et al.* (2011). El-Diasty and El- Kaseh(2009) found that total counts of aerobic bacteria was 6.1 X 10^5 counts/ml, while the mean coliform counts/ml was 7.0 X10^6 for examined raw milk. Also, Abou- Dawood(2005) found that coliform count in all raw buffalo's milk with an average 3.9x10^5 cfu/ml and TBC ranged between 10x10^6 to 20x10^7cfu/ml. Among the undesirable bacteria, the *Staph. aureus* was detected in all raw milk samples with an average of 5.3 log cfu/ml. Nearly similar findings were reported by Abou-Dawood *et al*. (2005) ,but higher than those reported by Godic Torkar and Golceger (2008).The Table also show that *E.coli* was detected in a percentage of 80 %in the examined raw milk , these results were higher than those found by Zeinhom and Abdel-Latef (2014) who revealed that *E. coli* was detected in a percentage of 26.7% and 16% in the examined raw market and bulk farm milk respectively. *B.cereus* was not detected in all samples. Data in the same table indicated that Salmonella was detected in 50% of the samples. These results was higher than that found by Abou-Dawood, *et al.,* (2005).

Moulds and yeasts were detected in all tested samples, the counts of moulds and yeasts ranged from 2.8 to 4.3 log cfu/ml with an average of 3.5 log cfu/ml. nearly similar findings were reported by El-Diasty and El- Kaseh (2008). They found that moulds and yeasts were detected in 80 % of raw milk samples, with respective mean values of 4.3 X10^5 cfu./ml.

As a result of the research, the samples of raw milk examined contained pathogen microorganisms. This may indicate that analyzed milk can contribute a potential risk for public health in the cases that it is consumed or used in the production of dairy products such as cheese, butter, cream and ice cream without being pasteurized or being subjected to a sufficient heat process. Microorganisms from soil, litter, feed, water, faces and other items in a farm environment commonly contaminate the surface of the udder and teats and the hair and skins of cows. From these sources they can get into the milk during milking. Unhygienic milking procedures and equipment used for milking, filtering, cooling, storing or distributing milk is also an important source of microorganisms. This situation is aggravated if the equipment is not properly cleaned and sanitized after use. Milk residues left on equipment and utensil surfaces provide nutrients to support the growth of many microorganisms, including pathogens (Bryan, 1983). Yilmaz *et al.,* (2009) reported that despite numerous studies that clearly show that raw milk can be contaminated by a variety of pathogens of known risk for human illness, people continue to consume raw milk. It is unlikely that countries that allow raw milk sales will change their regulations in the near future to prevent raw milk sales, and if they did, underground sales or other means to obtain raw milk would likely occur. Where raw milk is offered for sale, strategies to reduce risks associated with raw milk and products made from raw milk are needed. Developing uniform regulations including microbial standards for raw milk to be sold for human consumption, labeling of raw milk, improving sanitation during milking, and enhancing and targeting educational efforts are potential approaches to this issue. While many are opposed to regulatory intervention, regulations requiring that unpasteurized milk products meet process hygiene, food safety, and microbiological standards have worked effectively in other countries.

Microbiological evaluation of marketed Domiati cheese

Data of Table (2) reveals that the TBC of Domiati cheese ranged between 3.7 and 5.4 log cfu/gm, with an average 5.2 log cfu/gm. These results are less than those found by El-Sayed *et al.,* (2011).The Table (2) also show that the numbers of coliform bacteria ranged between 2.5to 4.4 log cfu/gm with an average of 3.6 log cfu/gm. *E.coli* and *E.coli* O157H7 were found in 20% and 4% of examined samples respectively . *Staph.aureus* was detected in 60% of Domiati cheese samples with an average of 4.4 log cfu/gm. *B.cereus* was not detected in all samples. Salmonella was found in 4% of samples. El-Sayed *et al.,* (2011) reported that

coliform group and *Escherichia coli* as fecal indicator contamination were implicated in 50 % of the samples with maximum counts of $3x10^4$ cfu/g in averages of $2x10^3$ cfu/g. The pathogenic *E.coli* O157H7 has been isolated from 19% of the total white cheese samples. Also the most hazardous enteric foodborne bacteria, *Salmonella spp*, has been only isolated from Domiati and Tallaga cheese varieties in 3 and 7 % of the samples, respectively. The maximum the counts of ~ 10^6 -10^7 cfu/g of *S. aureus and B. cereus,* in at least 3.3 % to 6.6 % of Domitai and Tallaga cheese samples. The higher that incidence rates of these pathogens in Domiati cheese samples pay much attention to that variety in particular from the side of hygienic quality. Our results are much higher than those found by Nour *et al.,* (1992) .They reported the presence of *Staph. aureus* in Domiati cheese samples collected from different sites in Egypt with nearly similar incidence close to that obtained by Eid and Eltalawy (2014). They examined 45 samples of Domiati, Tallaga, and Kareish cheese (15 samples of each) marketed in Gharbia governorate, Egypt, for the presence of *Staphylococcus aureus* organisms. The incidence of *Staph. aureus* were 13.3%, 26.6% and 26.6% in the examined cheese samples respectively, with a mean counts/g of 6.2×10^2 , 7.1×10^2 and 1.2×10^2 cfu/gm respectively. El Zayat, (1988) could not isolate *B. cerues* from any of 50 Domiati cheese sample collected from Ismailia governorate.Also , Heikal *et al.,* (2014) studied the bacteriological quality and safety of traditional white cheese in Tanta city , they found that high microbial counts reached to 6.80, 4.41 and 4.37 log cfu/g for total aerobe, coliforms and staphylococci respectively. *Bacillus cereus* was not detected in any cheese sample, while *E. coli*, *Salmonella* spp. and *Staphylococcus aureus* was found in 26.7%, 6.7% and 6.7% of examined cheese samples respectively.

Moulds and yeasts were detected in all tested samples, the counts ranged from 2.2 to 4.5 log cfu/gm with an average of 3.3 log cfu/gm. Nearly similar findings were reported by (El-Sayed *et al.,* 2011) .

Generally, cheeses showed high microbial counts reached to 5.2, 3.6 and 4.4 log cfu/gm for total aerobe, coliforms and staphylococci respectively. The microbial quality and safety of Egyptian varieties, white soft cheese, is the major area of concern for producers, public health authorities and consumers. It depends on the types of microorganisms introduced from raw milk, efficiency of processing and the hygienic practice applied in small or big dairy plant or informal producers. Handling of milks during cheese manufacture play an important role in the proliferation of microbial flora and consequently impair its utility and render the product unfit for human consumption .

Table 2: Microbiological properties of 50 Domiati cheese samples

Type of Microorganism	Positive samples		Counts (log cfu./gm)		
	No	%	Min.	Max.	Av.
Total bacterial count	50	100	3.7	5.4	5.2
Staph.aureus	30	60	3.3	5.4	4.4
Coliform group	40	80	2.5	4.4	3.6
E.coli	10	20	--	--	--
E.coli O157	2	4	--	--	--
Salmonella	2	4	--	--	--
Bacillus cereus	ND	ND	--	--	--
Yeasts & molds	50	100	2.2	4.5	3.3

Min. Minimum Max. Maximum Av. Average ND: Not Detected

Microbiological evaluation of marketed Kareish cheese

As shown in Table (3) the TBC of Kareish cheese ranged between 6.2 and 6.5 log cfu/gm.,with an average 6.3 log cfu/gm. The present data are in agreement with those obtained by El-Sayed *et al.,* (2011). Metwalli (2011) found that the TBC of Kareish cheese ranged between 50 and $21x10^7$ cfu/gm.

The Table (3) also show that the numbers of coliform bacteria ranged between 4.8to 6.3 log cfu/gm with an average of 5.8 log cfu/gm . *E.coli* and *E.coli* O157H7 were found in 33% and 6.7% of examined samples respectively . *Staph.aureus* was detected in 66% of Kareish cheese samples with an average of 5.3 log cfu/gm. *B.cereus* was not detected in all samples. Salmonella was found in 6.7% of samples.

Moulds and yeasts were detected in all tested samples, the counts of moulds and yeasts ranged from 4.5 to 5.7 log cfu/gm with an average of 5.5 log cfu/gm. nearly similar findings were reported by El-Sayed *et al.,* (2011). Metwalli (2011) found that the numbers of coliform bacteria ranged between 40to $290x10^3$ cfu/g with an average of $150x10^3$ cfu/gm,salmonella was not detected in all samples and *Staph.aureus* was detected in all Kareish cheese samples with an average of $2x10^5$ cfu/gm and moulds & yeasts were detected in all tested samples, the counts of ranged from 21to $6x10^3$ cfu/gm with an average of $4x10^3$ cfu/gm. The microbiological quality of Kareish cheese in our study indicates insufficient of sanitation during manufacture and handing this type of cheese . Kariesh cheese is sold uncovered and without container where the risk of contamination is high so it is considered as a good medium for the growth of different types of spoilage and pathogenic microorganisms. The implementation of ‘‘Good Manufacturing Practices’’ in the production of traditional cheese is fundamental for preventing contamination. The ‘‘Good Manufacturing Practices’’ are regulations,

concepts and procedures that aim at promotion and certification of quality in services, processes, and products (Lima *et al.,* 2008). It depends on the types of microorganisms introduced from raw milk, efficiency of processing and the hygienic practice applied in dairy plant. Handling of milk during cheese manufacture plays an important role in the proliferation of microbial flora and consequently impair its utility and render the product unfit for human consumption (Yousef *et al*., 2001, Leuschner and Boughtflower, 2002 and Aly and Galal, 2002).

Table 3: Microbiological properties of 30 Kareish cheese samples

Type of Microorganism	Positive samples		Counts (log cfu./ gm)		
	No	%	Min.	Max.	Av.
Total bacterial count	30	100	6.2	6.5	6.3
Staph.aureus	20	66	4.7	5.7	5.3
Coliform group	30	100	4.8	6.3	5.8
E.coli	10	33	--	--	--
E.coli O157	2	6.7	--	--	--
Salmonella	2	6.7	--	--	--
Bacillus cereus	ND	ND	--	--	--
Yeasts & molds	30	100	4.5	5.7	5.5

Min. Minimum Max. Maximum Av. Average ND Not Detected

Conclusion

In conclusion, it can be said that the raw milk sold in the study area was not satisfactory. It is confirmed by the high values of total bacterial count, Coliform, *Staph. aureus* and molds & yeasts counts. Presence of such higher counts causes deterioration in the quality of raw milk. Dairy producers know that the quality of milk and dairy products that consumers purchase depends in large part on the quality of milk they produce. The results demonstrate that the hygienic quality of white cheeses sold in markets in Cairo city was low and did not have enough assurance in terms of public health. To improve the safety of cheese efforts to raise awareness of the importance of hygiene barriers and raw milk quality as well as ensuring proper decontamination of processing equipment is essential to improve the safety of cheese for human consumption. It is important to initiate good hygiene practice (GHP) applications in farms to produce safe dairy products.

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