j.Egypt.net.med.Assoc 77, no 2. 209 – 223 (2017)

SOME MICROBIOLOGICAL QUALITY ATTRIBUTES OF KARISH CHEESE

By

Ola W. A.Hegab, Eman F. M. Abd El -Latif and Moawad A. A

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University; Giza, Egypt

ABSTRACT

Karish cheese is one of the most popular locally produced dairy products in Egypt. Seventy random samples of Karish cheese were collected from Cairo, Giza and Qaliubiya Governorates, representing: 35 samples of Karish cheese made from raw milk (small scale production) collected from street vendors, the other 35 samples representing dairy plants with different trade names (large scale production) collected from supermarkets. The collected samples were examined microbiologically and the results revealed that aerobic bacteria, proteolytic bacteria, yeast, mold, total staphylococci and coliforms were detected in different percentages and variable counts. On the other hand pathogens could be detected in small and large scale production as *S. aureus* and *E.coli*. The degree of acceptability of the examined samples vs. Egyptian Standards for Karish cheese was determined. The economic and public health significance as well as the suggestive preventive and control measures to monitor production of high quality and safe product were discussed.

Key words:

Karish, Kareish, Kariesh cheese, coliforms, *E. coli, S. aurus*, proteolytic, yeast and mould.

INTRODUCTION

Karish cheese is an acid coagulated white soft cheese. It is one of the most popular cheeses consumed in Egypt, especially in the countryside owing to its high protein, low fat, salt and price. Therefore; Karish cheese will be the most promising food to avoid the health problems associated with fat **(Kebary *et al*., 1997 and Korish and Abd Elhamid, 2012)**. In Egypt Karish cheese is manufactured using either skimmed raw milk (small scale production by farmers) or skimmed pasteurized milk (large scale commercial production in dairy plants). The small scale production usually exposes the product to high level of contamination as 28% of the out breaks involved cheese made from raw milk **(Fox *et al.*, 2000).** While large scale produced Karish cheese can be recontaminated from post pasteurization contamination, bad handing as well as bad storage **(Omar, 2006)**. The relatively high moisture content of Karish cheese makes it easily susceptible to microbial spoilage and growth of pathogens. High moisture in the cheese can allow any contaminants to multiply rapidly and cause spoilage, thus producing color and/or flavor defects which render the product of inferior quality. Therefore, proper sanitation, good packaging and refrigeration are mandatory for such cheese. Recently there are many reports of cheese borne food infections, and food poisoning, among these pathogenic microorganisms *E. coli* and enterooxigenic *S. aureus* **(Farkye and Vedamuthu, 2002).** This study was designed to examine Karish cheese sold in Egyptian markets (small and large scale production) microbiologically for aerobic plate count, proteolytic count, yeast count, mold count, total staphylococcal count, isolation and identification of *S. aureus*, enumeration, isolation and identification of coliforms and isolation and identification of *E.coli.*

MATERIAL AND METHODS

Collection of samples:

Seventy random samples of Karish cheese were collected from Cairo, Giza and Qaliubiya Governorates. Collected samples representing: 35 sample each of small scale production from street vendors and large scale production from supermarkets. Samples were taken under aseptic condition in sterile bags and transferred in an ice box to the laboratory.

Microbiological examination:

Preparation of food homogenate and decimal dilutions (APHA, 2004)

Aerobic plate count (CFU/g): (APHA, 2001)

Proteolytic count (CFU/g): (APHA, 2001)

Total yeast and mould count (CFU/g): (APHA, 2004)

Total staphylococci count (CFU/g): (ISO, 2003)

Isolation and identification of *Stapylococcus aureus*: (APHA, 2004)

Coliforms count (MPN/g): (APHA, 2004)

Isolation and identification of coliforms: (Silva *et al.,* 2013)

RESUL

TS

Table (1): Statistical analytical results of examined Karish cheese samples from small scale producers.

		Aerobic plate count CFU/g		Proteolytic count CFU/g		yeast count CFU/g		Mould count CFU/g		Total staphylococci count CFU/g		Coliforms count MPN/g	
	Incidence of positive samples	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
		35	100	28	80	35	100	22	62.9	35	100	35	100
Count/ g	Min	$11{\times}10^{6}$		$20{\times}10^{4}$		12×10^{3}		10×10^{2}		50×10^{2}		93	
	Max	$42{\times}10^{9}$		87×10^{8}		54×10^{7}		80×10^{5}		11×10^{6}		15×10^{7}	
	Mean	$90{\times}10^{8}$		14×10^{8}		97×10^{6}		20×10^{5}		10×10^{5}		11×10^{6}	
	± SEM	$21{\times}10^{8}$		4.2×10^{8}		19×10^{6}		5.9×10^{5}		3.9×10^{5}		5.3×10^{6}	

Table (2): Statistical analytical results of examined Karish cheese samples from large scale producers

		Aerobic plate count CFU/g		Proteolytic count CFU/g		yeast count CFU/g		Mould count CFU/g		Total staphylococci count CFU/g		Coliforms count MPN/g	
	Incidence of positive samples	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
		35	100	29	82.9	35	100	15	42.9	35	100	13	37.1
Count/ g	Min	60×10^{3}		10×10^{4}		25×10^{3}		100		100		4	
	Max	97×10^{7}		60×10^{5}		15×10^{6}		30×10^{3}		42×10^{4}		11×10^{2}	
	Mean	95×10^{6}		94×10^{4}		49×10^{5}		89×10^{2}		40×10^{3}		53×10	
	± SEM	37×10^{6}		27×10^{4}		7.6×10^{5}		27×10^{2}		16×10^{3}		14×10	

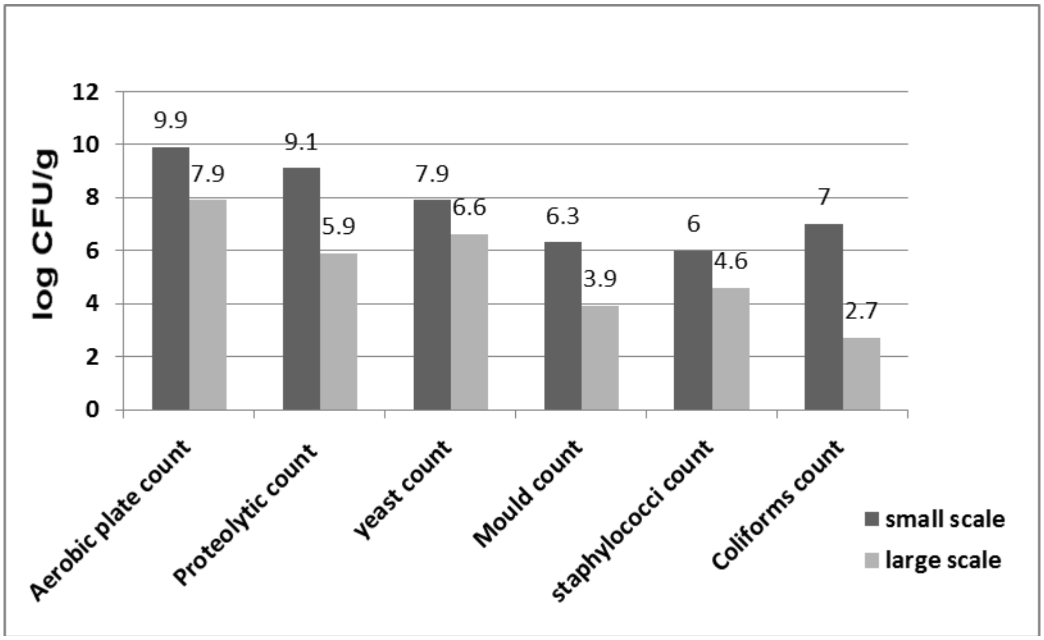

Fig. (1): Statistical analytical results of examined Karish cheese samples from small and large scale producers

Table (3): Frequency distribution of isolated coliform strains based on their biochemical identification (No. of isolates 105).

Isolated spp.	Small scale		Large scale		Total	
	No.	%	No.	%	No.	%
Escherichia coli	**4**	**7.7**	**3**	**5.7**	**7**	**6.7**
Klebsiella pneumoniae subsp. pneumoniae	**3**	**5.8**	**1**	**1.9**	**4**	**3.8**
Enterobacter intermedius	**10**	**19.3**	**26**	**49**	**36**	**34.3**
Yersinia frederiksenii	**2**	**3.8**	**4**	**7.5**	**6**	**5.7**
Klebsiella oxytoca	**2**	**3.8**	**2**	**3.8**	**4**	**3.8**
Klebsiella pneumoniae subsp. azoenae	**27**	**51.9**	**12**	**22.6**	**39**	**37.1**
Serratia fonticola	**4**	**7.7**	**2**	**3.8**	**6**	**5.7**
Klebsiella terrigena	**0**	**0**	**3**	**5.7**	**3**	**2.9**
Total	**52**	**100**	**53**	**100**	**105**	**100**

DISCUSSION

1-Aerobic plate count (CFU/g):

The most common method for enumerating bacteria present in raw milk and dairy products is referred to as the standard plate count (SPC). The SPC indirectly measures the number of aerobic bacteria that are capable of growth at 32°C **(D'Amico, 2014).** It is a useful indicator for monitoring the sanitary conditions applied during the production, collection, and handling of milk and dairy products, as large numbers of bacteria may indicate poor sanitation **(APHA, 2004)**. Results in (Table 1, 2) and Fig. (1) revealed that all the samples were positive for aerobic plate count either produced in small or large scale, with a minimum count of 11×10^6 and 60×10^3 CFU/g; the maximum count was 42×10^9 and 97×10^7 CFU/g; while the mean count was 90 × 108 ± 21 × 108 and 95 × 106 ± 37 × 106 CFU / g, respectively . Nearly similar results for small scale produced Karish cheese were obtained by **Baraheem *et al.* (2007),** while lower results were obtained by **Abd elsalam (2014), El-Nahas (2015)** and **Mamo *et al.* (2016)**. Results of large scale produced Karish cheese samples are nearly similar to those obtained by **Metwalli (2011) and Hassan and Gomaa (2016),** while higher results were obtained **by Baraheem *et al.* (2007) and Ibrahim *et al.* (2015).** Lower results were obtained by **Tudor *et al.* (2009) and Hussien *et al.* (2013)**. The Egyptian Standards of Karish cheese (1008- 4/2005) did not mention the acceptable level of aerobic plate count, but they obligate to pasteurize the cheese milk or any equivalent heat treatment with the addition of powerful starter culture. Dairy products should be regarded as unsatisfactory if they have a large population of microorganisms even if the organisms are not known to be pathogens. From the obtained results in this study, it is clear that even large scale produced cheese has high aerobic plate count as compared with small scale produced cheese owing to pasteurization of milk used in manufacture of such cheese, but it is need more attention to the sources of post pasteurization contamination, handling and storage to rise the product quality.

2- <u>Proteolytic count (CFU/g):</u>

Proteolytic microorganisms affect directly on the dairy product quality, it produces very active proteolytic and lipolytic enzymes which cause bitterness, putrefactive and rancid odors, liquefaction and gelatinization of curd (white, opaque curd turning translucent), slimy as well as mucous appearance of the curd surface. The proteolytic microorganisms include *Pseudomonas spp., Alcaligenes spp., Achromobacter spp., Flavobacterium spp and Bacillus spp.* which can survive pasteurization temperature and their presence may have related to foodborne illness **(Farkye and Vedamuthu, 2002)**. Concerning to results given in (Table 1, 2) and Fig. (1) Proteolytic organisms could be detected in 80, 82.9% of examined small and large scale production Karish cheese samples, respectively. The minimum count was 20×10^4 and 10×10^4CFU/g; the maximum count was 87×10^8 and 60×10^5CFU/g;

while the mean count was $14 \times 10^8 \pm 4.2 \times 10^8$ and $94 \times 10^4 \pm 27 \times 10^4$ CFU/g, respectively. Higher results were obtained by **Awad *et al.* (2005) and Ahmed (2016b)** for small scale produced Karish cheese, while lower result were reported by **Metwalli (2011)** for large scale produced Karish cheese. The high results of proteolytic contamination in small scale produced Karish cheese samples can be attributed to improper sanitation and absence of heat treatment beside bad handling and storage.

3- <u>Yeast count (CFU/g):</u>

Yeasts play diverse roles in affecting the quality and safety of dairy products they cause both economic and public health problems. The low pH and the nutritional profile of most cheeses are favorable for the growth of spoilage yeasts which can produce alcohol, CO_2 and off-flavors such as fermented, yeasty and fruity flavor. Common contaminating yeasts of cheesesinclude*Candida spp*.,*Kluyveromyces marxianus*,*Geotrichumcandidum*, *Debaryomyces hansenii*, and *Pichia spp*. **(Ledenbach and Marshall, 2009).** Although yeasts are rarely associated with foodborne infections, some studies have shown that, the presence of medically relevant yeast species in various cheeses, including *C. albicans, C. tropicalis*, *C. krusei,* and *C. glabrata* (**Banjara *et al.*, 2015**). Results presented in (Table 1, 2) and Fig.(1) showed that yeast count was detected in 100 % of both small and large scale produced Karish cheese samples with a minimum of 12×10^3 & 25×10^3 and a maximum of 54×10^7& 15×10^6, while the mean value was $97 \times 10^6 \pm 19 \times 10^6$ & $49 \times 10^5 \pm 7.6 \times 10^5$CFU/g, respectively. The obtained results of the small scale produced Karish cheese samples are nearly similar to those obtained by **Abd Elatif *et al.* (2016) and Abdel Hameed (2016)**. Lower results were reported by **Abd elsalam (2014), El-Leboudy *et al.* (2015) and Hassan and Gomaa (2016),** while higher results were obtained by **Soliman and Aly (2011)**, **Aly *et al.* (2012) and Ahmed (2016b)**. Lower results for large scale produced Karish cheese samples were obtained by **Soliman and Aly (2011) and Hussien *et al.* (2013)**. On studying the degree of acceptability of the examined small and large scale produced Karish cheese samples for yeast count vs. the Egyptian Standards (ES: 1008-4/2005) (not more than 400 CFU\g), there wasn't any of examined small and large scale produced Karish samples were acceptable. The high incidence of yeast may be due to inadequate hygienic measures during production, improperly stored (dusty) containers or the use of bad quality raw materials **(Varnam and Sutherland, 2009).**

4-<u>Mould count (CFU/g):</u>

Growth of spoilage moulds on cheese is a problem that has economic and public health effects. Moulds form pigmented colonies (black, green or dark brown) on the cheese surface. They impart a musty, mouldy odor with production of discoloration, and liquefaction of the curd due to their powerful proteolytic enzymes. Some moulds could produce toxins in cheese that could present a health risk to the consumer having mutagenic and teratogenic effect, the resulting symptoms range from skin irritation to immunosuppression, birth defects, neurotoxicity or death. The most common moulds found on cheese are species of the genera *Penicillium*, *Aspergillus, Alternaria, Mucor, Fusarium, Cladosporium and Geotrichum* **(Farkye and Vedamuthu, 2002 and Nsofor and Frank, 2013)**. The given results recorded in (Table 1, 2) and Fig. (1) revealed that moulds could be detected in 62.9 and 42.9% of small and large scale produced Karish cheese samples, with a minimum value of 10×10^2 & 100; a maximum value of 80×10^5&$30 \times 10^3$and a mean value of $20 \times 10^5 \pm 5.9 \times 10^5$ & $89 \times 10^2 \pm 27 \times 10^2$CFU/g for small and large scale produced Karish cheese samples, respectively. Nearly similar findings of small scale produced Karish cheese samples were reported by **Ibrahim *et al.* (2015).** Lower results were obtained by **Seifu (2013)**, **Abdel Hameed (2016) and Younis *et al.* (2016)**, while higher results were obtained by **Ashor (2001) and Ahmed (2016b)**. Nearly similar findings of large scale produced Karish cheese samples were reported by **Hussien *et al.* (2013).** Yeasts and moulds generally enter cheese as contaminants from the air, or improperly stored (dusty) containers. The obtained high count of moulds in the examined cheese samples generally correspond to poor cleaning practices and the use of un-hygienic techniques or inadequate storage conditions. Air filtration, UV irradiation, and regular sanitation with fungicides can aid in the control of airborne contaminants **(D'Amico, 2014)**. Based on the **Egyptian Standards (1008-4/2005)** for mould count, the degree of acceptability of the examined samples showed that 37.1% and 57.1% of the examined small and large scale produced Karish cheese samples, respectively, were considered acceptable (less than 10 CFU/g).

4-<u>Total staphylococcal count (CFU/g) and *S. aureus*:</u>

Staphylococci are impossible to be eradicated from the environment, *S. aureus* can be found in soil, water, and air; it is also lives as a normal inhabitant in humans and animals. Food handlers are frequently the main source of food contamination in staphylococcal outbreaks; however, equipment and environmental surfaces also can be other sources. Staphylococci are able to grow at pH range between 4.5 and 9.3 so it can survive in cheese environment. **(FDA, 2012 and EL-Kholy *et al.*, 2014).** Several *Staphylococcal species*, including both coagulase-negative and coagulase-positive strains, have the ability to produce highly heat-stable enterotoxins that cause gastroenteritis in humans. *S. aureus* is a food-born pathogen which is capable of causing staphylococcal food poisoning, toxic shock syndrome, pneumonia; post-operative wound infection, and nosocomial bacteremia. Staphylococcal food poisoning occurs due to ingestion of food containing preformed heat-stable enterotoxins produced by this microorganism. The intoxication dose of *S. aureus* enterotoxin is less than 1.0 microgram. This toxin level is reached when *S. aureus* populations exceed 10^5organisms/g in food. This level is indicative of unsanitary conditions in which the product can be rendered injurious to health. Symptoms appear within 1 to 7 hours after contaminated food is eaten which include nausea, abdominal cramping, vomiting, and diarrhea. In more severe cases, dehydration, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur **(FDA, 2012 and EL-Kholy *et al.*, 2014).** Data recorded in (Table 1, 2) and Fig.(1) showed that staphylococci were found in all examined small and large scale produced Karish cheese samples, with minimum of 50×10^2 & 100, a maximum of 11×10^6 & 42×10^4, while a mean value of $10 \times 10^5 \pm 3.9 \times 10^5$ & $40 \times 10^3 \pm 16 \times 10^3$ CFU/g for small and large scale produced Karish cheese samples, respectively. The obtained results of small scale produced Karish cheese samples are nearly similar to **Atta (2003) and Alsayed (2010).** Lower results were reported by **Aly *et al.* (2012)**, **Awad (2016) and Mamo *et al.* (2016),** while higher results obtained by **Abd elsalam (2014)**.The data presented in this study confirm presence of *S. aureus* in 40% and 22.9% of small and large scale produced Karish cheese samples, respectively, based on coagulase test and thermostable nuclease test. Lower incidence of *S. aureus* for small scale produced Karish cheese was reported by **Alsayed (2010)**, **Eid and Eltalawy (2014) and El-Bastawesy (2016)**. Higher finding obtained by **El-Leboudy *et al.* (2015)**, **Ibrahim *et al.* (2015) and Hassan and Gomaa (2016)**. Higher incidence for large scale produced Karish cheese samples was reported by **Kolta (2011)**. According to the Egyptian Standards (1008-4/2005) Karish cheese should be free from *S. aureus*. 60% & 77.1% of small and large scale produced Karish samples were acceptable. Presence of high level of staphylococci in cheese is usually due to unhygienic handling of the product and/or an extensive contamination by personnel possibly involved in cheese making and marketing as humans are the common carriers.

5-<u>Coliforms count (MPN/g):</u>

Coliforms rarely cause defects in cheese made of pasteurized milk because they are not heat-resistant, however; recontamination of pasteurized milk or bad handling and storage can allow these organisms to grow and cause defects. While in Karish cheese which made traditionally from raw milk, coliforms are found due to fecal contamination or biofilms on milking equipment. Maintaining low coliform levels in cheese milk (<10 CFU/ml) is critical, as coliforms can multiply rapidly, when pH and temperature are favorable causing flavor defects including yeasty, putrid, and gassy flavors **(Walstra *et al.,* 1999 and D'Amico, 2014)**. Data represented in (Tables 1,2) and Fig.(1) showed that coliforms were detected in 100% and 37.1% of examined small and large scale produced Karish cheese samples, respectively. Coliforms count was ranged from 93 to $15 \times 10^7$with and average of $11 \times 10^6 \pm 5.3 \times 10^6$ in small scale production samples MPN/g. While in large scale production samples it ranged from 4 to 11×10^2 with an average of $53 \times 10 \pm 14 \times 10$ MPN/g. Nearly similar results of small scale produced Karish cheese samples were reported by **Meshref and Hassan (2009)**, **El-Prince *et al.* (2010) and Abd elsalam (2014).** Lower results were reported by **Awad (2016), Hassan and Gomaa (2016) and Mamo *et al.* (2016),** while higher results were obtained by **Yilma *et al.* (2007), Melkamsew *et al.* (2012) and Ahmed (2016b)**.For the large scale production Karish cheese higher findings were obtained by **Baraheem *et al.* (2007)** and lower ones were reported by **Hussien *et al.* (2013).** On studying the degree of acceptability of the examined small and large scale produced Karish cheese samples vs. **Egyptian standards (ES:1008-4/ 2005)** for coliforms count (not more than 10 CFU/g), 100% and 74% of the examined small and large scale produced Karish samples were unacceptable.

<u>Isolated coliforms:</u>

According to the biochemical identification, the obtained isolates were identified as: *E.coli* (7.7, 5.7%), *Kleb. pneumoniae subsp. pneumoniae* (5.8, 1.9%), *Ent. intermedius* (19.3, 49%) *Yersinia frederiksenii* (3.8, 7.5%)*, Kleb. oxytoca* (3.8, 3.8%)*, Kleb. pneumoniae subsp. azoenae* (51.9, 22.6%), *Serratia fonticola* (7.7, 3.8%) and *Kleb. terrigena* (0, 5.7%) in small and large scale produced Karish cheese samples, respectively (Table 3). *Klebsiella spp*. can be found in unpasteurized milk. *Kleb. pneumoniae* may be considered as an Entero-pathogen; since this organism produces heat-stable and heat-labile enterotoxins and has been associated with a few foodborne cases. *Enterobacter spp.* are opportunistic pathogens widely distributed in nature and have been found in dairy products **(FDA, 2012).** Similar findings were obtained by **Melkamsew *et al.* (2012),Mohammed *et al.*(2013) and Nosir(2014)**.*E. coli* is a bacterium that is present in the gastrointestinal tract of man and dairy animals. Milk can become contaminated with *E.coli* directly from animal feces or indirectly via contaminated farm and dairy parlor environments, equipment, and workers. The public health hazard and incidence of *E.coli* has been emphasized by many authors as a result of increasing numbers of outbreaks of *E. coli* in dairy products. *E. coli* strains can cause a variety of diseases including dysentery, hemolytic uremic syndrome (HUS), urinary tract infection (UTI), septicemia, pneumonia, and meningitis **(Fratamico *et al.*, 2002 and Bhunia, 2008).**According to virotypes pathogenic *E. coli* are classified into six groups: Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Entero-invasive *E. coli* (EIEC), Entero-aggregative *E.coli* (EAEC), and diffusely adhering *E. coli* (DAEC). Enteropathogenic and Enterotoxigenic *E. coli* are associated with infant diarrhea as well as traveler's diarrhea in individuals traveling to developing countries. Enter invasive *E. coli* results often in bloody watery diarrhea, mucus typical of shigella infections. Enter aggregative *E. coli* produce a cytotoxin which is believed to cause diarrhea in young children and adults. Enterohemorrhagic *E. coli* causing bloody diarrhoea and Haemolytic Uremic Syndrome (HUS) **(Bhunia, 2008, Dedeić-Ljubović *et al.,* 2009 and D'Amico, 2014)**. The serological identification of the suspected isolates of *E. coli* by slide agglutination test showed that 3 isolate out of 4 in small scale produced Karish cheese samples were positive and their antigenic formula were+poly II O128: K 67, +poly I O44:K 74 and+ poly IIIO118: K- , while in large scale produced Karish cheese samples 1 isolate out of 3 was positive with antigenic formula + poly I O44: K 74. The strain O44: K 74 varityped as EPEC, ETEC and EAEC. While O128:K67 and O118: K- are EPEC, EHEC and ETEC **(Dedeić-Ljubović *et al.,* 2009)** *E.coli* could be isolated in small scale produced Karish cheese samples by **Ibrahim *et al.* (2015), Ahmed (2016a) and El-Bastawesy (2016)**. While **Abdel-Salam (2014) and Ahmed (2016b)** couldn't detect it. In large scale produced Karish cheese samples **Hussien *et al.* (2013) and Mohammed *et al.* (2013)** could not isolate *E.coli.* **Egyptian standards (ES: 1008-4/ 2005)** mentioned that *E.coli* shouldn't be present in Karish cheese, 91.4% and 97.1% of the examined small and large scale produced Karish samples were acceptable. It is clear from the obtained results that Karish cheese may possess a public health hazard as it is found to be a source of pathogenic *E.coli* so regulatory measures as good manufacturing practices should be implemented for ensuring microbiological safety of Karish cheese during manufacture.

CONCLUSION

The microbiological quality of large scale Karish cheese samples was superior to small scale Kariesh cheese samples. The average counts in all tested microbial parameters were higher in small scale Karish cheese samples; therefore, to safeguard consumers from being infected and to obtain Karish cheese of good keeping quality, recommendations should be applied to use pasteurized skimmed milk in manufacturing of Karish cheese and strict hygienic measures should be adopted during manufacturing and storage.

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