

Full Length Research Paper

# Exploring microbiological standards and quality attributes of Karish cheese from Egypt

Sameh Awad

Department of Dairy Science and Technology, Faculty of Agriculture, Alexandria University, Egypt. E-mail: sameh111eg@yahoo.com or sameh.awad@alexu.edu.eg. Tel: 020 3 5903915.

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Some traditional fermented dairy products like Egyptian Karish cheese and traditional fermented milk (Laban Zeer) are manufactured in farmhouses following traditional techniques using raw milk without heat treatment or addition of selected starter cultures, these products are generally designed as “artisanal”. The representative Karish samples were analyzed for their content of total microbial, *Enterobacteriaceae*, yeast, molds, *Staphylococci* coagulase positive, *Salmonella* spp., *Listeria* spp., in addition to detection of *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens* and *Campylobacter* spp. using classical methods. The physico-chemical properties of collected samples showed that the average of protein, fat, moisture and acidity were in accepted range with that reported in literatures and Egyptian standard. Some lactic acid bacteria (*Lactococcus lactis* supsp. *lactis*, *Lactobacillus delbrueckii* subsp. *lactis* and *Enterococcus faecium*) were isolated and identified from fresh karish cheese using classical methods followed by rep-PCR. *Lactococcus garvieae* was detected in fresh samples using TTGE but could not be detected using classical methods. The results of microbiological analysis showed the presence of high numbers of *Enterobacteriaceae* that ranged from  $\log_{10}$  2.6 cfu/g to  $\log_{10}$  3.5 cfu/g, *Bacillus* ssp. ranged from  $\log_{10}$  4.2 cfu/g to  $\log_{10}$  5.6 cfu/g, *Staphylococci* counts are ranged from  $\log_{10}$  1.8 cfu/g to  $\log_{10}$  2.7 cfu/g, faecal enterococci count ranged from  $\log_{10}$  3.5 cfu/g to  $\log_{10}$  5.8 cfu/g. Yeasts and molds count ranged from  $\log_{10}$  2.7 cfu/g to  $\log_{10}$  3.5 cfu/g. *B. cereus*, *L. monocytogenes*, *C. perfringens*, *Shigella*, *Salmonella*, *Escherichia coli*  $\beta$ -glucuronidase positive and *Campylobacter* spp. were not detected in all the examined samples. The count of undesirable microorganisms indicated the poor hygienic practice of traditional Karish cheese production; starting with using poor quality of raw milk and processing under uncontrolled environments. This would require using clean raw milk, controlled manufacturing steps and selection of the appropriate starter culture for the fermentation.

**Key words:** Karish cheese, microbiological analysis, safety.

## INTRODUCTION

Karish cheese is traditional soft type cheese commonly made in Egyptian villages at farmhouses. It is an acid

dairy product and made from defatted milk, the cheese is traditional made by leaving the raw milk for coagulation by natural flora and then collecting the fat layer. The fat in dry matter and the moisture content in Karish cheese should not exceed 10 and 75%, respectively (Egyptian Standard 2000/4-1008).

Microbial communities of traditional fermented dairy products are complex and insufficiently characterized. Microbial diversity is considered essential for the sensory properties of traditional cheeses. However, some members of these complex communities may also constitute a health risk (Montel et al., 2014). Consequently, studying and understanding this particular ecosystem is of great interest from both public health and economic perspectives (Irlinger et al., 2015).

Karish cheese is usually produced under artisanal conditions using raw milk without heat treatment. Numerous microorganisms, including bacteria, yeasts and molds constitute the complex ecosystem in traditional Karish cheese were analyzed using classical methods. The analysis results showed extremely high numbers of *Staphylococcus* spp. ( $3.6 \times 10^5$  cfu/g), coliform ( $18.9 \times 10^3$  to  $6.9 \times 10^7$  cfu/g); faecal enterococci ( $8 \times 10^6$  cfu/g), *Enterobacteriaceae* (in the range of  $1.01 \times 10^6$  to  $1.34 \times 10^8$  cfu/g) which was reported by El Leboudy (1998), Said and Fahmy (1991), El-Kholy (1989) and Tawfek et al. (1988).

In the last decades, culture-independent techniques were used to study the total microbial communities in fermented dairy products. Results of culture-independent techniques have greatly improved our understanding of their composition, dynamics and activities (Irlinger and Mounier, 2009). When compared with culture-based methods, PCR is faster, more sensitive, more specific and enables detection of sub-dominant populations, even in the absence of a selective enrichment medium and in the presence of other (dominant) populations. Moreover, it allows detection of dead cells or viable but non-cultivable cells (Delbès et al., 2007; Irlinger et al., 2015). The origin, safety and functional role of microbial communities in Karish cheese making are still not well understood. The microbial communities, present on Karish cheese, largely could not be completely defined by classical methods. For this purpose, semi-systematic approach based on genetic profiling by temporal temperature gradient electrophoresis (TTGE) has been used by El-Baradei et al. (2005) to describe the bacterial ecosystem of some samples of Karish cheese, but the analysis of cheese during storage has not been done. So, the aims of this work were to evaluate the lactic acid bacteria (LAB) involved in the cheese fermentation and cheese safety by defection of the pathogenic bacteria. Evaluation of lactic acid bacteria was carried out using both culture dependent (enumeration, isolation and genotypic characterization of bacteria strains on selective medium) and independent (PCR-TTGE analysis) methods. Undesirable microbial groups (e.g., *Bacillus*, coliform and

*Enterobacteriaceae*) and pathogenic bacteria (for example, *Staphylococcus aureus* coagulase positive, *Bacillus cereus*, *Listeria monocytogenes*, *Clostridium perfringens*, *Shigella*, *Salmonella*, *Escherichia coli*  $\beta$ -glucuronidase positive and *Campylobacter* and) were also analyzed by classical methods. Also, texture profile analysis and physico-chemical properties were also examined.

## MATERIALS AND METHODS

### Samples

Fifteen (15) samples, of traditional Karish cheese made using raw milk, were collected from local markets in different Governorates of Egypt (Alexandria, Behira, Kafer El Sheick, Domiata, Gharbia). The fresh cheeses were transferred into ice box to the laboratory and stored at refrigerator temperature ( $5 \pm 1^\circ\text{C}$ ) for 15 days.

### Physico-chemical analysis

Total protein was determined by Kjeldahl (AOAC, 2003) and fat content was measured by Gerber method (AOAC, 2003). Titratable acidity was expressed as percentage of lactic acid content of cheese by weight. pH value was measured in well-mixed ground cheese samples. Moisture analyzer (Mettler Toledo Model HR73) was used to determine the moisture content. Chloride meter (Jenway, England, UK) was used to determine sodium chloride in cheese samples.

### Texture profile analysis

Karish cheese cubes (50 x 50 x 50 mm) were prepared and placed in plastic cups, sealed (to prevent dehydration) at temperature of  $10 \pm 0.5^\circ\text{C}$  prior to analysis. A two-bite penetration test was performed using the Texture Analyzer (TA1000, CNS-Farnell, England) operated at a crosshead speed of  $1 \text{ mm s}^{-1}$  and compressed to 50%. Hardness, cohesiveness, springiness and chewiness were evaluated in triplicate according to the definitions given by Bourne (1978).

### Microbiological analysis

Undesirable bacterial group were plated on selective agar media as follows:

1. Brilliant green agar for *Enterobacteriaceae* (ISO 21528-2-2004)
2. Violet red bile agar for coliform group (Difco 1998)
3. Xylose lysine deoxycholate agar (XLD, Biolife, Italy) was used for *Salmonella* and *Shigella* spp. (Mahon and Manuselis, 1995).
4. Oxford agar (Biolife, Italy) for *L. monocytogenes* (Anon, 1995 and ISO 11290-1/A1:2004).
5. Baird-parker medium for *Staphylococcus* spp. (ISO 11867, 1997)
6. Mannitol-egg yolk-polymyxin agar, (MYP, Biolife, Italy) for *B. cereus* (ISO 7932, 2004).
7. Modified-*Campylobacter* blood free selective agar base (modified-CCDA, Oxoid, England) for *Campylobacter* spp. (ISO 10272-1, 2006).
8. Sulfite-cycloserine agar (SC, Biolife, Italy) for *Clostridium perfringens* (ISO 7937, 2004).
9. Oxytetracycline glucose yeast extract agar for yeast and molds

(O.G.Y.E agar, LAB, United Kingdom) (ISO 6611, 2004).

10. Tryptone bile X-glucuronide agar (TBX, Biolife, Italy) for *E. coli*  $\beta$ -glucuronidase positive (ISO, 16649-1, 2001).

#### Isolation and identification of lactic acid bacteria by Rep-PCR

Lactic acid bacteria were isolated and pre-identified according to the methods described by El Soda et al. (2003). The isolates were identified using rep-PCR as described by Mohammed et al. (2009). The pellet of each isolate was obtained by centrifugation of 1.6 ml of fresh culture in log phase at 5000 xg for 10 min at 10°C and then the pellet was washed twice with sterile deionized water and stored overnight at 4°C. Total DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wiscon). DNA concentration of each isolate was adjusted to 25 ng/ $\mu$ L, to which 0.3  $\mu$ M BOXAIR primer (5'CTACGGCAAGGCGACGCTGACG-3') was added (Biolegio BV, Nijmegen, The Netherlands). Ready-To-Go PCR beads (Amersham Biosciences) that included 200  $\mu$ M deoxynucleoside triphosphate, 2.5 U puRe Taq DNA polymerase (Amersham Biosciences), 10 mM Tris-HCl pH 9, 50 mM KCl, 1.5 mM MgCl<sub>2</sub> and sterile deionised water to reach a final volume of 25 $\mu$ L were also added. PCR amplification was performed in a Flexigene Thermal Cycler (Techne, Duxford, Cambridge, UK) according to the polymerase chain reaction (PCR) programme (De Urza et al., 2000). PCR products were separated by electrophoresis at 50 V on a 2% (w/v) agarose gel (Amersham Biosciences) and DNA was detected by UV trans-illumination after staining with ethidium bromide (10 mg/mL). The molecular sizes of amplified DNA fragments were estimated by comparison with a 100 bp DNA ladder (Promega) and were photographed using the Digimage System (Major Science, Pan-Chiao City, TaipeiHsien, Taiwan). The Rep profiles were processed using Gel ComparII version 5.00 software (Applied Maths, Kortrijk, Belgium). Pearson correlation coefficient was used and UPGMA was done to cluster the different groups.

#### Temporal temperature gradient gel electrophoresis (TTGE)

Each Karish cheese sample (5 g) was dissolved in 40 ml of sterile trisodium citrate 2% and homogenized by Ultra-Turrax mechanical blender at 18000 rpm for 1 min until the solution was opaque. 50 mg of Pronase (Protease, from *Streptomyces griseus*- SIGMA-ALDRICH) and 100  $\mu$ l of  $\beta$ - mercaptoethanol were added to each sample. This was followed by 3 h incubation at 50°C. Both cheese fats and proteins were removed by centrifugation at 10,000 rpm/15min at 4°C. The pellet of bacteria was washed twice with TES buffer (25 mM tris-HCl, 0.1 M EDTA, 25% (wt/vol) sucrose pH 8) and centrifuged. The cells were resuspended in 480  $\mu$ l of TE buffer and 120  $\mu$ l of lysozyme followed by incubation at 37°C for 1 h and centrifugation at 10,000 rpm/10min at 4°C. The supernatant was removed and total DNA was extracted from bacterial cells (pellet) as described using Wizard Genomic DNA purification Kit with the manufacturer's (Promega, Madison, Wisconsin).

Forty five different bacterial species or subspecies of bacteria with low G+C genomes were selected for reference strains setup belonging to the genera *Lactobacillus*, *Lactococcus*, *Enterococcus*, *Leuconostoc*, *Streptococcus* and *Pediococcus*.

#### Nested-PCR

The V3 region of the 16S rRNA gene is the substrate for PCR amplification. The extracted DNA was amplified by two successive PCR amplifications as described by Ogier et al. (2002).

#### TTGE analysis

PCR products obtained from V3 region amplifications were subjected to TTGE analysis. TTGE was performed by using the Dcode Universal mutation detection system (Bio Rad) that were 16 x 16 cm x 1 mm according to the method described by Ogier et al. (2004).

#### Statistical analysis

Data reported are the average of three measurements. The SAS statistical analysis software package (SAS, 1999) was used for analysis of variance. Differences were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Physical and chemical analysis of Karish Cheese

The physico-chemical properties of Karish cheeses are summarized in Table 1. The pH value was ranged from 4.21 to 4.65 in fresh cheeses and it decreased from 3.98 – 4.35 after 15 days of storage, while the average of acidity was 1.56% of fresh cheese and increased to 1.75% after 15 days of storage. The moisture content ranged from 66.8 to 73.4% with an average of 69.5% in fresh cheese and decreased to 66.5% after 15 days of storage, this shows fat and protein content which increased after 15 days of storage, while the salt content was not changed during storage. The fat in Karish cheeses was variable in most of the samples; the minimum fat level was 3.4%, while the maximum fat level was 6.4% with an average of 5.2% in fresh samples. The protein level of cheese ranged from 15.4 to 19.4% in fresh samples with average of 17.8%. The data of physico-chemical analysis of Karish cheese was similar with that reported by Todaro et al. (2013) and Egyptian Standard (1008/4/2000). Various factors affect the chemical composition and physical properties of Karish cheese like, this include the milk type, season of milk production, fat separation method used, micro flora of the milk and/or starter addition, additives in the cheese milk, storage time and temperature. The composition of Karish cheese is in the range of 17.7 to 22.5% protein, 2.2 to 5.8% fat and 64.8 to 71.7% moisture (Todaro et al., 2013).

### Texture profile analysis of Karish cheese

The texture profile analysis including hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness of Karish cheese samples are shown in the Table 2. There was a relationship between chemical composition and texture profile analysis as the hardness increased after 15 days of storage with decrease in the moisture content. The hardness and chewiness are both increased at 15 days of storage due to the increase in protein content and decrease in the moisture content and

**Table 1.** Physico-chemical analysis of traditional Karish cheese.

Storage/days	pH value		Acidity (%)			Fat (%)			Protein (%)			Moisture			Salt (%)		
	Min	Max	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave
1	4.21 <sup>a</sup>	4.65 <sup>a</sup>	1.25 <sup>b</sup>	1.71 <sup>b</sup>	1.56 <sup>b</sup>	3.4 <sup>b</sup>	6.4 <sup>b</sup>	5.2 <sup>b</sup>	15.4 <sup>b</sup>	19.4 <sup>b</sup>	17.8 <sup>b</sup>	66.8 <sup>a</sup>	73.4 <sup>a</sup>	69.5 <sup>a</sup>	0.2 <sup>a</sup>	0.4 <sup>a</sup>	0.3 <sup>a</sup>
15	3.98 <sup>d</sup>	4.35 <sup>d</sup>	1.45 <sup>a</sup>	1.98 <sup>a</sup>	1.75 <sup>a</sup>	3.6 <sup>a</sup>	6.6 <sup>a</sup>	5.5 <sup>a</sup>	16.4 <sup>a</sup>	20.1 <sup>a</sup>	18.9 <sup>a</sup>	64.5 <sup>d</sup>	71.5 <sup>d</sup>	66.5 <sup>d</sup>	0.2 <sup>a</sup>	0.4 <sup>a</sup>	0.3 <sup>a</sup>

Each value is the average of three replicates. Min: the minimum value of 15 samples, Max, is the maximum value of 15 samples, Ave: is the average of 15 samples; <sup>a-b</sup> Means within a column with no common superscript differ ( $P < 0.05$ ).

**Table 2.** Texture profile analysis of traditional Karish cheese.

Days	Hardness (g)	Cohesiveness ratio	Adhesiveness (gs)	Springiness (mm)	Gumminess (gs)	Chewiness (g)
0	128	0.78	5.2	7.4	99.84	738.81
15	174	0.78	5.2	7.4	135.72	1004.32

Each sample has analyzed three times and the present value is the average of 15 samples.

pH value. Many factors influence texture development of Karish cheese. These include those factors that affect the curd moisture content (temperature of coagulation and drain of whey), cheese composition, pH, interactions between casein and serum proteins, Ca content, ionic strength, salt content, and manufacturing protocol, especially rate and extent of acid development. Fat content in the cheese is responsible for its many desirable functional and texture. In addition, decreasing moisture content might result in decrease in the level of free moisture in cheese; this increased the hardness (Awad, 2011).

#### Microbiological analysis of Karish cheese samples

##### *Enterobacteriaceae* and coliform bacterial count

The results of coliform bacterial count of fresh

Karish samples on VRB agar ranged from  $\log_{10}$  3.4 to  $\log_{10}$  5.2 cfu/g with an average of  $\log_{10}$  4.6 cfu/g (Table 3). *Enterobacteriaceae* count of fresh Karish samples on Brilliant Green agar ranged from  $\log_{10}$  2.6 to  $\log_{10}$  3.5 cfu/g, there was no bacterial growth in only one sample. The Egyptian Standard for Karish cheese (1008/4/2000) recommended that the coliform bacteria should be not more than 10 cfu/g. The high level of *Enterobacteriaceae* and coliform bacteria in Karish cheese indicated the poor hygienic practice during processing of the cheese. The results are in agreement with Aboul Kheir et al. (1985). They found *Enterobacteriaceae* with average of  $1.01 \times 10^6$  cfu/g in more than 85% of Karish cheese samples. *E. coli* was in about 81% of examined samples. While El-Kholy (1989) found higher count of *Enterobacteriaceae* in fresh Karish cheese with an average of  $1.34 \pm 0.93 \times 10^8$  cfu/g and *E. coli* was detected in 23% of samples. Karish cheese samples collected from different

localities in Fayoum city, Egypt, contain high level of coliform and faecal enterococci at average level of  $18.9 \times 10^3$  and  $8 \times 10^6$  cfu/g of cheese, respectively (Tawfek et al., 1988). High levels of coliform bacterial group were also reported in Karish cheese by Metwalli (2011).

##### *Escherichia coli* $\beta$ -glucuronidase positive

Tryptone-bile-x-glucuronide (TBX) agar was used for the detection of *E. coli*. After the period of incubation, there were no bacteria growth (blue or blue green colonies) in all tested Karish samples. Svanberg et al. (1992) and Svanberg (1996) reported that lactic acid-fermented gruels inhibited the proliferation of Gram-negative pathogenic bacteria including toxicogenic *E. coli*. The Egyptian Standard for Karish cheese (1008/4/200) recommended that the cheese should be free from *E. coli*.

**Table 3.** Microbiological analysis of Karish samples.

Number of days	Log <sub>10</sub> CFU/g on VRA			Log <sub>10</sub> CFU/g on Brilliant Green			Log <sub>10</sub> CFU/g on MYP agar			Log <sub>10</sub> CFU/g on SF agar			Log <sub>10</sub> CFU/g on Baird Parker agar			Log <sub>10</sub> CFU/g on MRS agar			Log <sub>10</sub> CFU/g On O.G.Y.E agar		
	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave	Min	Max	Ave
1	3.4	5.2	4.6	2.6	3.5	3.2	4.2	5.6	5.2	3.5	5.8	4.9	1.8	2.7	2.6	6.7	9.8	8.5	2.7	3.5	3.4
15	2.5	4.4	3.2	1.8	2.5	2.2	1.5	2.2	1.8	2.5	4.5	4.0	0	2.6	1.3	5.9	7.8	7.6	3.4	3.8	3.6

Three to six dilutions of each sample were analyzed. Min: the minimum value of 15 samples, Max, is the maximum value of 15 samples, Ave: is the average of 15 samples.

### Detection of *Shigella* and *Salmonella* spp.

Desoxycholate and xylose-lysine-desoxycholate (XLD) agars are intermediate selective media and are preferred media to isolate *Shigella* and *Salmonella* spp. *Shigella* colonies on XLD agar medium are translucent and red (alkaline). The results of this study confirmed the absent of *Shigella* and *Salmonella* in all examined samples.

### Bacterial count on MYP agar

The microbial count relating to *Bacillus* ssp. of Kishk samples on MYP agar are variable and the counts on the medium ranged from log<sub>10</sub> 4.2 to log<sub>10</sub> 5.6 cfu/g with an average of log<sub>10</sub> 5.2 cfu/g (Table 3).

It is obvious from the results that all the examined samples contained *Bacillus* ssp. This may be due to the use of raw milk in Karish cheese making and the contamination of the milk from the soil and during the Karish cheese processing. There was significant reduction of bacilli count of Karish cheese samples after 15 days; these is mostly related to the low pH in Karish cheese. Wong and Chin (1988) reported that lactate (0.1 M) completely inactivated multiplication of *B. cereus* at pH 5.6. In general, due to the lower pH value (~4.2- 4.6), fermented

milks is not a suitable environments for the majority of spoilage-causing bacteria (Samarzija et al., 2012).

The presumptive colonies on MYP medium were tested for hemolysis on blood agar base No 2. α-Haemolysis was not observed in all Karish samples, while β-hemolysis was represented in 4 out of 15 samples.

### *Staphylococci* count on Baird Parker agar and detection of Coagulase-positive *Staphylococci* count

Results of *Staphylococci* count for kishk samples showed that the *Staphylococci* counts on Baird Parker agar ranged from log<sub>10</sub> 1.8 to log<sub>10</sub> 2.7 cfu/g (Table 3). The presumptive colonies were tested for coagulase using dry-spot staphylect, after inoculation in brain heart infusion broth and streaking colonies on Baird Parker agar. Typical colonies on Baird Parker medium are black to gray, brilliant and convex and surrounded by a clear zone, which can be partially opaque and confirmed by coagulase test using dry-spot staphylect. Coagulase-positive *Staphylococci* were detected in five out of fifteen samples. This is also related to poor hygienic practice in cheese manufacturing, these samples are not within Egyptian Standard for Karish cheese (1008/4/

2000) as it is recommended that the cheese should be free from Coagulase-positive *Staphylococci*. *S. aureus* is usually the dominant pathogens in traditional Karish cheese. This microbe was found in about 10% of examined karish cheese samples by Abou-Donia (1984). *S. aureus* was isolated from 66 and 78% of the examined Karish cheese samples by Tawfek et al. (1988) and by Said and Fahmy (1991), respectively. *S. aureus* count was 3.6 x10<sup>5</sup> cfu/g and 41% of the isolates were coagulase positive (Said and Fahmy, 1991).

### Detection of *C. perfringens*

Sulfite-cycloserine (SC) agar was used for *C. perfringens* count. After incubation, the black were calculated. There was no growth in 12 out of 15 tested Kishk samples and presumptive colonies were inoculated into fluid thioglycollate medium then five drops of thioglycollate medium. Cultures were further examined for the production of gas and the presence of a black colour in LS medium. Durham tubes more than one-quarter full of gas and tubes having a black precipitate are considered positive for the occurrence of *C. perfringens*. From the results, the occurrence of *C. perfringens* was none in the examined samples.

### **Lactic acid bacterial count**

Enterococci were enumerated on SF agar. Lactobacilli were enumerated on MRS agar and lactococci were enumerated on M17 agar. Enterococci count ranged from  $\log_{10}$  3.5 to  $\log_{10}$  5.8 cfu/g with an average of  $\log_{10}$  4.9 cfu/g. Concerning, Lactobacilli ranged from  $\log_{10}$  6.7 to 9.8 cfu/g with an average of  $\log_{10}$  8.5 cfu/g, while lactococci were in the range of  $\log_{10}$  6.3 to 7.8 cfu/g with an average of  $\log_{10}$  6.9 cfu/g (Table 3). The lactic acid bacterial count is related to the raw milk used in making the Karish cheese.

### **Detection of *L. monocytogenes***

The presumptive colonies were streaked onto tryptone soya yeast extract broth. Colonies were confirmed with appropriate morphological test (Gram positive, slim and short rods) and some biochemical tests such as catalase test (3%), the positive catalase reaction indicated *L. monocytogenes*. The obtained results from all Karish samples confirmed all the examined samples were free of *L. monocytogenes*.

### **Detection of *Campylobacter* spp.**

The representative samples were analyzed for the occurrence of *Campylobacter* spp. The results of all the samples confirmed that the detected colonies in the selective medium did not belong to *Campylobacter* spp. Svanberg et al. (1992) and Svanberg (1996) reported that lactic acid-fermented gruels inhibited the proliferation of Gram-negative pathogenic bacteria including *Campylobacter jejuni*.

### **Yeasts and molds**

The results of yeasts and molds count of fresh Karish samples on oxytetracycline glucose yeast extract agar ranged from  $\log_{10}$  2.7 to  $\log_{10}$  3.5 cfu/g with an average of  $\log_{10}$  43.4 cfu/g, the average of yeasts and molds increased to  $\log_{10}$  3.6 cfu/g after 15 days. The Egyptian Standard for Karish cheese (1008/4/200) recommended that the yeasts should not exceed 400 cfu/g while the molds should not exceed 10 cfu/g. The high level of yeasts and molds in Karish cheese indicated the poor hygienic practice during processing of the cheese. Yeasts and molds were also presented at high level:  $7.1 \times 10^6$  cfu/g in Karish cheese samples (Abou-Donia et al., 1975).

### **The diversity of lactic acid bacteria using TTGE and rep-PCR**

The TTGE database of standard strains was used to

identify major bacterial populations present in 5 samples of traditional Karish cheese at 1 day and after 15 of manufacturing as shown in Table 4. The obtained results indicated that the *L. lactis* and *L. delbrueckii* subsp. *lactis* are the predominant species in fresh and 15 days old cheese. Only these both species were detected in all fresh samples by both methods "dependent and independent". *L. delbrueckii* subsp. *lactis* was also detected by TTGE in all five samples after 15 days of storage, while the *L. Lactis* was detected in only four samples out of five after 15 days of storage.

*L. garvieae* was detected by only TTGE in 3 fresh samples out of 5; it was detected in same samples after 15 days, while *L. garvieae* could not be detected by dependent methods in all the five samples. *Enterococcus faecium* was detected in 4 fresh out of 5 samples by TTGE, while it was detected in only 2 samples by classical methods.

*L. plantarum* was detected by TTGE in 3 fresh samples (2, 4 and 5) but it was detected in only one sample by rep-PCR (2), while it was detected in 4 samples after 15 days of storage (1, 3, 4, 5). *L. acidophilus* was also detected by TTGE and rep-PCR in 3 samples (1, 3, 5), *L. fermentum* was detected by TTGE and rep-PCR in 3 samples (3, 4, 5). *L. acidophilus* and *L. fermentum* was detected by TTGE in same fresh and 15 days old samples. *S. thermophilus* was detected in 3 fresh samples by TTGE and in only 2 samples by rep-PCR. *Leuconostoc mesenteroides* was detected in 3 samples by TTGE and in one sample by rep-PCR while *Leuconostoc lactis* was detected in 2 samples by TTGE and in one sample by rep-PCR. In comparing between directed and indirect methods in detecting the bacterial culture during storage of traditional Karish cheese, it is clear that more strains could be detected using TTGE than direct method. *L. garvieae* was detected in fresh samples by using TTGE and could not be detected using directed method. *S. thermophilus* was detected in 3 samples by TTGE while it detected in only one sample by rep-PCR. All the strains that were detected by rep-PCR were also detected by TTGE except *L. lactis* and *L. mesenteroides* which were detected in one sample by only rep-PCR. Moreover, *L. garvieae*, which is a dominant population in Karish cheese by TTGE-PCR, has never been detected on lactococcal counting plates, same finding was also found in traditional Egyptian Domiati cheese (El-Baradei et al., 2007) and in traditional, panish, blue-veined Cabrales cheese (I orez and Mayo 2006). TTGE confirmed 10 different lactic acid bacterial species that are present in fresh karish cheese and some strains could be isolated and identified by traditional analyses of microbiota using cultivation on specific or non-specific growth agars. The weakness of phenotypic methods include poor reproducibility and discriminatory power, laboriously investigations, and the ambiguity of some techniques caused by complex growth conditions (Cocolin et al., 2007). It is well documented

**Table 4.** Lactic acid bacteria biodiversity of Karish cheese during storage.

Cheese age	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
	TTGE	Rep-PCR								
<i>Lc. garvieae</i>	+	-	+	-	-	-	+	-	-	-
<i>E. faecium</i>	+	+	-	-	+	+	+	-	+	-
<i>Lb.del. subsp.lactis</i>	+	+	+	+	+	+	+	+	+	+
<i>Lb. plantarum</i>	-	-	+	+	-	-	+	-	+	-
<i>Lc. Lactis</i>	+	+	+	+	+	+	+	+	+	+
<i>Lb acidophilus</i>	+	+	-	-	+	+	-	-	+	+
<i>Lb. fermentum</i>	-	-	-	-	+	+	+	+	+	+
<i>Ln. mesenteroides</i>	+	-	+	-	-	-	-	+	+	-
<i>Ln. lactis</i>	+	-	+	-	-	-	-	-	-	+
<i>Str. thermophilus</i>	+	-	-	-	+	+	-	-	+	-
<i>Lc. garvieae</i>	+		+		-		+		-	
<i>E. faecium</i>	+		-		+		+		-	
<i>Lb.del. subsp. lactis</i>	+		+		+		+		+	
<i>Lb. plantarum</i>	+		-		+		+		+	
<i>Lc. Lactis</i>	-		+		+		+		+	
<i>Lb. acidophilus</i>	+				+		-		+	
<i>Lb. fermentum</i>	-		-		+		+		+	
<i>Ln. mesenteroides</i>	+		+		-		-		+	
<i>Ln. lactis</i>	+		+		-		-		+	
<i>Str. thermophilus</i>	-		-		+		-		+	

Gels were analyzed using database with 140 reference strain.

that stressed or injured cells could not be recovered in selective media and that cells present in low numbers are very often inhibited by other microbes (Hugenholtz et al., 1998). For these reasons, it is important to have methods that allow monitoring of the microbial populations without cultivation by culture-independent methods (Cocolin et al., 2004; Mangia et al., 2015). The culture-independent methods allowed us to confirm the dominant bacterial community of Karish cheeses that are almost identified in each sample “*L. lactis subsp. lactis*, *L. delbrueckii*

*subsp. lactis*, *L. acidophilus* and *L. lactis*”. These bacteria may play the main role in the fermentation and organoleptic properties of Karish cheeses because of their common presence in the tested samples.

### Conclusion

Traditional cheeses like Karish are widely consumed and also contribute to the Egyptian culture. Microbial diversity is considered essential

to the sensory richness and safety of Karish cheeses. Although, traditional Karish cheese is made from raw milk without the addition of any starters, most of the bacteria identified in this study as dominant are lactic acid bacteria. However, some members of these complex communities were identified as responsible for cheese flavor defects (coliform group, *Enterobacteriaceae*) and some other constitute a health risk (coagulase-positive *Staphylococci*). The results of this study confirmed the improvement in Karish cheese manufacturing which is necessary to obtain a safe

and homogenous product.

## Conflict of interests

The authors have not declared any conflict of interests.

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