

## Original Research Article

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**Microbiological Quality of Kareish cheeses**

Tawfik Abdel Rahman Elbassiony<sup>1</sup>; Khaled Mohamed El-Khawas<sup>1</sup>, Nadia Mohamed Mostafa<sup>2</sup> and Rania Mohamed Ewida<sup>3</sup>

<sup>1</sup>Department of Food Hygiene (Milk Hygiene), Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

<sup>2</sup>Animal Health Research Institute, Dokki, Giza, Cairo 12618, Egypt

<sup>3</sup>Department of Food Hygiene (Milk Hygiene), Faculty of Veterinary Medicine, New Valley University, New Valley 72511, Egypt

\* Corresponding Author

Rania Mohamed Ewida, 3Department of Food Hygiene (Milk Hygiene), Faculty of Veterinary Medicine, New Valley University, New Valley 72511, Egypt.

r\_ewida@vet.nvu.edu.eg

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**Abstract**

This study aimed to evaluate kareish cheese sold in two cities in Egypt (New Valley and Cairo). A total of 105 kareish cheese samples were collected from New Valley, Cairo and different cheese factories (35 samples for each). The samples were examined for the occurrence of some food poisoning microorganisms as well as their quality. All samples were submitted for physical, chemical, and microbiological examination. The physical examination revealed that 100% of the examined samples were normal in color, odor, and consistency. The mean values of salinity and pH were  $2.0 \pm 0.26$  and  $4.4 \pm 0.04$ , respectively. The positive % of coliforms, *E. coli*, and molds & yeasts were 42.8, 30.5, and 89.5%, respectively with the mean count  $3.45 \pm 0.22$ ,  $1.92 \pm 0.22$ , and  $4.62 \pm 0.13$  cfu/g, respectively. Moreover, *Staphylococcus aureus*, *Salmonella spp.* and *Listeria monocytogenes* could not be detected in the examined kareish cheese samples. The results clarified that the high risk for humans that may be occurred by consumption of contaminated kareish cheese. Hygienic measures must be taken during the manufacture of this type of cheese.

**Keywords:** *E. coli*, Kareish cheese, *L. monocytogenes*, *Salmonella spp.*, *Staph. aureus*.

**Introduction**

Kareish cheese is one of the soft white cheeses of ancient Egyptian. It is the most popular type of cheese in Egypt due to its high nutritive value with a low price besides it has low-fat content because the manufacture of this type depends on the use of raw skim milk. It is made mainly by acid coagulation of skimmed milk by natural microorganisms present in the milk or by the addition of rennet to the raw or pasteurized skim milk. The two methods of manufacture are still unhygienic, the final products are contaminated with various types of microorganisms (Ahmed and El-Bassiony, 1977 and Deeb et al., 2004). Those microorganisms may lead to food poisoning diseases and/or the final product with inferior quality (Todaro et al., 2013). Many food poisoning microorganisms could be found in Kareish cheese as coliforms. The presence of coliforms in milk products indicates the inferior quality of the milk used in manufacture (Yabaya and Idris, 2012). *E. coli* is the most serious coliform found

in milk and milk products. *E. coli* is normally inhabiting the intestine of animals and humans, but some strains have pathogenic or toxigenic virulence genes that make them virulent for animals and human (Malik and Memona, 2010). *Staph. aureus* is another group of bacteria that is normally inhabitant in the nose, throat, and skin of animals and humans. *Staph. aureus* can produce enterotoxins that cause food poisoning diseases (Orwin et al., 2003). Moreover, *Salmonella spp.* and *L. monocytogenes* have been reported as food poisoning pathogens which cause a serious public health hazard in many countries with raw and pasteurized milk or related products as butter and cheese (De Buyser et al., 2001; Heghbeart et al., 2003; Mazurek et al., 2004 and Olsen et al., 2004). Molds and yeasts are widely present in a natural environment (Kurtzman et al., 2011). They can grow in a wide range of temperature, pH, and humidity, thus lead to many economic losses in dairy products as discoloration, gas production, and taint-flavor (Arias et al., 2002). Also, some molds can produce

mycotoxins as aflatoxins which have a carcinogenic effect on human health. Considering all the public health significance of some food poisoning microorganisms as well as economic losses, the present study was planned to deal with physical, chemical, and microbiological examination for enumeration, isolation, and identification of some food poisoning microorganisms from marketable Kareish cheese sold in New Valley Governorate and Cairo City, Egypt.

## Materials and Methods

### Collection of samples

A total of 105 samples of homemade (Falahy) and factory-manufactured Kareish cheese samples were collected for examination from New Valley and Cairo markets (70 Falahy samples 35 each from New Valley and Cairo markets and 35 factory-manufactured samples). The samples were fresh or pickled kareish cheese.

The collected samples were transferred directly to the laboratory in an icebox under complete aseptic conditions without undue delay and then subjected to the following examination.

### Sensory examination

Samples were examined for changes in color, odor, and consistency by the analyst panel (members of Food Hygiene Department, Animal Health Research Institute, Giza, Cairo, Egypt).

### Chemical examination

#### Determination of Salinity (Sodium chloride): (A.O.A.C., 2019)

3 g sample was moistened in 300 ml flask with excess silver nitrate solution (25ml 0.1N depending on NaCl contents of the sample). 15 ml nitric acid was added and boiled until the cheese was dissolved (10 min). Pot. permanganate solution was added in portions, boiled after each addition until  $KMnO_4$  color was disappeared and the solution became colorless or nearly then 25 ml water was added and boiled 5 min cooled, diluted to about 150 ml, 25 ml ether was added and shaken and titrated till light brown color, ml of the pot. thiocyanate used was subtracted from ml of silver nitrate added and calculated the difference as NaCl %.

With 10 g sample each: ml 0.1 N = 0.058 % NaCl

#### Determination of pH (Harold et al., 1981).

About 10 g of cheese sample was homogenized well with 100 ml previously boiled distilled water (water temperature 25°C) and left to stand for 10 minutes. Using pH meter (Jenway, 3310), each sample was measured three times; the pH value was recorded as an average of the three readings.

### Microbiological examination

Preparation of serial dilutions (ISO 6887-5:2010):

25 g of cheese samples were placed in a stomacher bag with 225 ml of sodium citrate solution and stomached for 1 min. This mixture produced 10-1 dilution. Ten-fold serial dilutions were made using peptone water.

#### Coliform count (US-FDA, 2020):

From the previously prepared homogenate, aseptically two 1 ml aliquots of each dilution were inoculated to sterile Petri dishes, about 10 ml Violet Red Bile Agar (VRBA) tempered to 48°C were poured into plates, and let to solidify, overlaid with 5 ml VRBA, and incubated for 24 h at 35°C. Purple-red colonies that are 0.5 mm or larger in diameter and surrounded by a zone of precipitated bile acids are the typical characters for coliform. For confirmation, colonies have been transferred to a tube of BGLB broth. Incubated at 35°C and examined at 24 - 48 h for gas production.

#### *E. coli* count (ISO, 16649/2 - 2001):

From the previously prepared homogenate, aseptically 1 ml aliquot of each dilution was inoculated to sterile petri dish then 10 ml Tryptone Bile X-Glucuronide (TBX) agar were poured, then incubated at 44.5°C for 24 h. Characteristic colonies of *E. coli* were bluish colonies with a bluish halo zone.

Triple Sugar Iron (TSI), Indol test, Methyl Red Test, Voges-Proskaur test and Citrate utilization test were used as biochemical tests to identify the isolated strains (A.P.H.A., 2004).

#### *Staphylococcus aureus* count (US-FDA, 2016):

From the previously prepared homogenate, aseptically 1 ml sample suspension was transferred to 3 plates of Baird-Parker agar, distributing 1 ml of inoculum equitably to 3 plates (i.e., 0.4 ml, 0.3 ml, and 0.3 ml). The inoculum was spread over the surface of agar plates, using a sterile bent glass streaking rod. Plates were incubated for 48 h at 35-37°C. Colonies of *S. aureus* were circular, smooth, convex, moist, 2-3 mm in diameter on plates, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone, and frequently with an outer clear zone. The suspected colonies were confirmed for coagulase activity using Dry Spot Staphylofect Plus.

**Isolation of *Salmonella* spp. (ISO 6579:2017):**

Pre-enrichment in non-selective liquid medium:

A 25 g of cheese samples were placed in a stomacher bag with 225 ml buffered peptone water, stomached for 1 min, and incubated for  $18 \pm 3$  h at  $37^\circ$  C.

**Enrichment in selective liquid media:**

Exactly 0.1 ml of pre-enrichment broth culture was added to 10 ml Rappaport Vassiliadis broth with soya (RVS broth) then incubated at  $41.5^\circ$ C for  $24 \pm 3$  h, also 1 ml of pre-enrichment broth were added to Muller-Kauffmann Tetrathionate/novobiocin both (10 ml MKTTn) and incubated at  $37^\circ$  C for  $24 \pm 3$  h.

**Plating out & Identification:**

A loopful from the selectively enriched broth was streaked onto the surface of previously prepared xylose lysine deoxycholate agar (XLD agar) and Brilliant Green agar (BG). Inoculated plates were incubated at  $37^\circ$  C for  $24 \pm 3$  h. Plates were examined for suspected colonies that appear as pink with or without black center on XLD agar and red to pink-white colonies surrounded by a red zone on Brilliant Green agar. The suspected colonies were subjected to biochemical tests (using API 20).

**Isolation of *Listeria monocytogenes* (US-FDA, 2017):**

Exactly 25 g of cheese samples were stomached in 225 Buffered Listeria Enrichment Broth and incubated at  $30^\circ$  C for 24 -48 h. Then LEB culture was streaked onto Oxford agar and Aloa agar then Incubated at  $35^\circ$  C for 24-48 h. Listeria colonies are black with a black halo on oxford agar and blue with a halo on Aloa agar. The suspected colonies were subjected to biochemical tests (using microbact 12 L).

**Mold and yeast count (ISO 21527-1:2008):**

From the previously prepared homogenate, aseptically 0.1 ml was inoculated onto the center of two plates containing Dichloran Rose Bengal Chloramphenicol agar media. Inoculated plates were incubated aerobically in an upright position at  $25^\circ$  C for 5 days.

**Results**

Data recorded in Table (1) revealed that all the examined kareish cheese samples had normal color, odor, and consistency. Data illustrated in Table 2 declared that the mean value of NaCl content in the examined Kareish cheese samples were  $3.9 \pm 0.83$ ,  $1.3 \pm 0.39$ , and  $2.2 \pm 0.16$  in the samples obtained from New Valley, Cairo, and cheese factories, respectively.

**Table (1):** Sensory examination of the examined Kareish cheese samples

Parameter	Region	New Valley	Cairo	Factory	Total
	Color	Normal	100%	100%	100%
Abnormal		0	0	0	0
Odor	Normal	100%	100%	100%	100%
	Abnormal	0	0	0	0
Consistency	Normal	100%	100%	100%	100%
	Abnormal	0	0	0	0

**Table (2):** Statistical analysis of Salinity and pH for the examined Kareish cheese samples

Parameter	Region	New Valley	Cairo	Factory	Total
	Salinity	Min	2.1	0.7	1.0
Max		6.1	5.2	3.2	6.1
Mean		3.9 <sup>a</sup>	1.3 <sup>b</sup>	2.2 <sup>c</sup>	2.0
SE*		0.83	0.39	0.16	0.26
pH	Min	3.9	4.1	3.8	3.8
	Max	5.3	4.7	4.6	5.3
	Mean	4.6 <sup>a</sup>	4.3 <sup>a</sup>	4.2 <sup>a</sup>	4.4
	SE*	0.05	0.03	0.06	0.04

\* SE: Standard Error

There are significance differences ( $P < 0.05$ ) between means having different letters.

There was significant difference ( $P < 0.05$ ) among the cheese samples. On the other hand, pH values ranged from (4.4<sub>-</sub>+0.04), however, there was no significant difference ( $P \sim 0.05$ ) between the different cheese samples collected from different regions.

The data summarized in Table (3) showed that the mean counts of coliform in samples collected from New Valley, Cairo, and cheese factories were (3.15<sub>-</sub>+0.47) log cfu/g, (3.73<sub>-</sub>+0.18) log cfu/g, and (3.40<sub>-</sub>+0.49) log cfu/g, respectively. It was noticed that there are no significant differences ( $P \sim 0.05$ ) between cheese samples in different regions. On the other hand, the positive samples were 42.8% of the total collected samples.

**Table (3):** Statistical analysis of coliforms count log cfu/g of the examined Kareish cheese samples\*

Parameter	Region	New Valley	Cairo	Factory	Total
	Minimum		1.00	2.60	1.00
Maximum		4.99	5.60	5.80	5.80
Mean		3.15 <sup>a</sup>	3.73 <sup>a</sup>	3.40 <sup>a</sup>	3.45
SE**		0.47	0.18	0.49	0.22
Positive No.		11	17	17	45
Positive %		31.4	48.5	48.5	42.8
Un satisfactory No. ***		11	17	17	45

Un satisfactory %	31.4	48.5	48.9	42.8
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\* For positive samples only  
 \*\* SE: Standard Error  
 \*\*\* According to ES (2010)  
 There are significant differences ( $P < 0.05$ ) between means having different letters.

It was obvious that in Table (4), the percent of the positive samples about 30.5% of the total collected samples for *E. coli*. Moreover, the mean count of the examined New Valley cheese samples (0.97<sub>-</sub>+0.27) log cfu/g was significantly higher than that of the mean of the examined Cairo cheese samples (2.39<sub>-</sub>+0.11) log cfu/g and cheese factories (2.79<sub>-</sub>+0.89) log cfu/g.

**Table (4):** Statistical analysis of *E. coli* count log cfu/g of the examined kareish cheese samples\*

Parameter	Region			
	New Valley	Cairo	Factory	Total
Minimum	0.30	1.78	1.30	0.30
Maximum	2.71	3.23	4.82	4.82
Mean	0.97 <sup>a</sup>	2.39 <sup>b</sup>	2.79 <sup>b</sup>	1.92
SE <sup>**</sup>	0.27	0.11	0.89	0.22
Positive No. <sup>***</sup>	10	16	6	32
Positive %	28.5	45.7	17.1	30.5
Un satisfactory No. <sup>***</sup>	10	16	6	32
Un satisfactory %	28.5	45.7	17.1	30.5

\* For positive samples only  
 \*\* SE: Standard Error  
 \*\*\* According to ES (2010)  
 There are significant differences ( $P < 0.05$ ) between means having different letters.

*Staph. aureus* count was lower than the level of counting, moreover, *Salmonella spp.*, and *L. monocytogenes* could not be isolated from the different types of the examined Kareish cheese samples.

As illustrated in Table (6), the mold and yeast count in the examined Kerish cheese samples collected from Cairo and New Valley were higher than that collected from manufactures cheese samples. It was obvious that the positive samples were 89.5% the highest value was in New Valley 100% followed by Cairo at 94.2% and factory-manufactured samples 74.2%.

**Table (5):** Statistical analysis of mold and yeast count log cfu/g of the examined kareish cheese samples\*

Parameter	Region			
	New Valley	Cairo	Factory	Total
Minimum	3.72	1.00	1.00	1.00
Maximum	5.80	6.26	7.00	7.00
Mean	4.85 <sup>a</sup>	4.84 <sup>a</sup>	3.75 <sup>b</sup>	4.62
SE <sup>**</sup>	0.07	0.21	0.45	0.13
Positive No.	35	33	26	94
Positive %	100	94.2	74.2	89.5
Un satisfactory No. <sup>***</sup>	35	32	22	89
Un satisfactory %	100	91.4	62.8	84.7

\* For positive samples only  
 \*\* SE: Standard Error  
 \*\*\* According to ES (2010)  
 There are significant differences ( $P < 0.05$ ) between means having different letters.

### Discussion

Regarding the sensory analysis, the results were similar to Mahmoud (2020), showed instead of carried out that all the examined pickled Kareish cheese (100%) had good flavor, appearance, and color, while in the examined fresh kareish cheese 90% had good flavor, 84% of the examined samples with good body and texture and 88% of the samples had good appearance and color. While, the salinity results were higher than Mahmoud (2020), who examined fresh Kareish cheese sold in New Valley Governorate, while the obtained results were lower than the same author when examined the pickled kareish cheese samples. Salt acts as a preservative by reducing the water activity which leads to a decrease or prevents the growth of microorganisms and germination of its spores besides it play important role in flavor enhancer (Abdalla and El-Zubeir, 2006). According to coliforms counts results, the higher results were shown by Meshref and Hassan (2009), El-Leboudy et al. (2015), and Ahmed et al. (2018). Concerning the incidence of *E. coli*, nearly similar results were obtained by Nosir et al. (2014); Ibraim et al. (2015), Mohammed (2020), and Sayed-Ahmed (2020), while Meshref and Hassan (2009); Basha et al. (2012), Elbagory et al. (2015), Salem et al. (2016) and Kamal et al. (2017) reported higher incidence percent; 56, 86, 37.5, 48 and 45 %, respectively. According to the Egyptian Standard (2010) which stated that coliforms count must not be higher than 10 cfu/g (1 log cfu/g); 43% of total samples, 49% of Cairo and factories samples and 31% of New Valley samples are rejected

due to the high coliforms count than the accepted limit. Kareish cheese contaminated by *E. coli* may be attributed to inefficient heat treatment of the raw milk used in cheese manufacture or post-pasteurization contamination during transportation, handling, and sale of Kareish cheese (Ewida and Hussein, 2018). The incidence of *Staph. aureus* was similar to that carried by Ghada et al. (2002), they couldn't detect *Staph. aureus* in kareish cheese samples collected from the plants, while they were isolated from the examined home or farmers made kareish cheese samples in only one sample (2%). Moreover, the obtained incidence of *Staph. aureus* was lower than that the incidence obtained by El-Leboudy et al. (2015), Kamal et al. (2017), Abo El-Makarem (2018), ElShall (2019), Mohammed (2020), and Sayed-Ahmed (2020). On the other side, Sayed-Ahmed (2020) failed to isolate *Salmonella spp.* and *L. monocytogenes* from the examined Kareish cheese samples, while El Sayed et al. (2011) isolated *L. monocytogenes* in a percentage of 13.3 from the examined Kareish cheese samples. The low pH of Kareish cheese effect in growth most of bacteria as *Staph. aureus*, *Salmonella*, and *L. monocytogenes*, thus lead to the medium not favorable to the growth of these bacteria. On the other side, acidic pH cheese is a good medium for the growth and multiplication of other types of microorganisms as the molds and yeasts grow and multiply in high numbers. The mold and yeast incidences were higher than El-Leboudy et al. (2015) and Mohammed (2020), who recorded the incidence of molds and yeasts were 76.6 and 70%, respectively, from the examined Kareish cheese samples collected from Dakahlia Governorate and Sohag Governorate, Egypt. Regarding the mean count of molds and yeasts reported by Ahmed et al. (2018) was 6.2 log cfu/g higher than that reported in this study. While the lower results were carried out by Hakim et al. (2013). The accepted limit for mold and yeast counts in Kareish cheese according to Egyptian Standard (2010) is equal to or less than 410 cfu/g (2.61 log cfu/g). According to this limit, 85% of the total samples, 100, 91 and 63% of New Valley, Cairo and factories samples, respectively are rejected due to high mold and yeast counts than the accepted limit. The growth of molds and yeasts in cheese may induce many defects in these products as off-flavor, color defect, and actual rots (Mislivec et al., 1992). Besides, the molds and yeasts can produce mycotoxins, this type of toxin leads to many diseases in humans and

animals such as cancer and human food poisoning (El-Leboudy et al., 2015).

## Conclusion

From the obtained results kareish cheese is a vehicle for transamination of some food poisoning microorganisms to humans. Therefore, it is recommended to use high-quality raw milk for the manufacture of kareish cheese. Moreover, pasteurization of raw milk must be applied to all milk used in kareish cheese manufacture.

## Conflict of interest

The authors declare that they have no competing interest.

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