# THE MICROBIOLOGICAL QUALITY AND PHYSICOCHEMICAL ATTRIBUTES OF EGYPTIAN TRADITIONAL SA'EEDI KISHK

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## SUMMARY

The objectives of this study were to assess the microbiological quality, the physicochemical attributes and mineral content of Sa'eedi Kishk (SK). Forty samples were collected from four provinces in Upper Egypt for this study. Total bacterial, lactic acid bacteria and enterococci counts, as a natural microflora, were monitored. Coliforms, Listeria sp., coagulase positive staphylococci and yeast & mold counts were pursue in SK. One hundred isolates of lactic acid bacteria (LAB) were isolated and identified by phenotype and biotype methods. Also, the physicochemical attributes of SK include determination of moisture, total solids, crude protein, crude fat, ash content, total fiber, total carbohydrates, and pH values were evaluated. Meantime, nine minerals, i.e., P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu were determined. Overall results indicate that SK samples were free from potential food borne pathogens, highly nutritious, contain many minerals and overloaded with unique lactic acid bacteria strains.

*Keywords:* Sa'eedi Kishk,, (LAB), Kishk microbiology, Kishk physiochemical attributes, Kishk minerals.

## **INTRODUCTION**

Kishk is fermented buttermilk-wheat mixture originated during Pharonic period (3200- 322 B.C.) in Upper Egypt (also known as Sa'eed) and being hitherto produced (Abou-Donia, 2008). Kishk is a balanced food with excellent preservation quality and well adapted to hot climates without refrigeration (Blandino et al., 2003). The low moisture content (less than 10%), low pH of 4.2 and the non-hygroscopic nature of Kishk are crucial reasons play a significant role to extend the shelf life of such traditional food (El-Gendy, 1983; Abou-Donia, 1984; Attia & Khattab, 1985 and Tamime & O'conner, 1995). Similar products like Sa'eedi Kishk are also know in other middle east countries with the same name in Syria, Lebanon and Jordan (Tamime & O'conner, 1995). Other names are given to fermented milk-wheat mixture similar to SK such as kushuk (Iraq), Kashk (Iran), Tarhana (Turkey), Trahanas (Cyprus & Greece), Tarhonya (Hungary) and Talkuna (Finland) (Georgala, 2013). The preparation of Kishk in Egypt remains until now a household or a home art performed by tillers wives either for their family consumption or for sale in the nearby markets. The product is parboiled wheat known as 'belila' and strained fermented buttermilk 'laban zeer' mixture in a ratio of two to one respectively

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(Eldamhougy, 1972). The microbiology of SK was documented over the years in several published reports (Abd El Malek & Demerdash, 1970; El-Gendy, 1983; Abou-Donia, 1984; Attia & Khattab 1985; El-Nawawy et al., 2012 and Awad et al., 2013).

Kishk is considered as part of the rich food heritage of Egypt with unique nutritional benefits (Awad et al., 2013). Unfortunately, it is subjected to extinction due to changes in lifestyle emerged nowadays in rural districts of Upper Egypt, the habitat of Kishk, according to (El-Nawawy et al., 2012 and Saleh, 2013). Therefore, the objective of the present paper was to revisit by research such traditional dairy food from different aspects. Firstly, the microbiological and physicochemical attributes of Kishk from four principal provinces in Sa'eed (Assiut, Beni Swif, Fayoum, & Giza) were subjected for a survey study and the results obtained are the core of the present paper. Secondly, the functional properties and sensory evaluation of Kishk will be a subject of a separate investigation.

## MATERIAL AND METHODS

## Kishk Sample collection

Forty samples of Kishk (~500g each) were procured from different local markets in provinces located in Upper Egypt. Those were Assiut (A), Beni-Swif (B), Fayoum (F) and Giza (G). Kishk samples collected from province (A) were characterized by its round shape (Fig. 1). However, samples obtained from provinces (B, F & G) were of irregular shape as appeared in (Fig. 2).

## Microbiological examination

## Microbiological media

Seven microbiological tests were monitored in Kishk samples included total viable count (TVC), Lactic acid bacteria count (LAB), enterococci count (EC), coliform count (CC), coagulase positive staphylococci count (CPSC), Listeria Sp. count (LC), Yeast and mold count (Y & M). Table (1) summarized the specific media and methods used.





Fig. (1): Kishk round shape

Fig. (2): Kishk irregular shape

Test	Media	Method	Reference	
Total viable count	Plate - count agar	Standard plate	Laird et al (2004)	
(TVC)	(Hi-media)	count	Lund et ul. (2004)	
Lactic acid bacteria count	MRS- agar			
(LAB)	(Oxoid)	Direct plate		
<i>Enterococcus</i> count (EC)	Kanamycin azide agar (Hi-media)	count	Frank et al. (2004)	
Coliform count (CC)	VRBA (Merck)	Modified VRBA procedure	Davidson et al. (2004)	
Coagulase+ve staphylococcus aureus count (CPSC)	S-110 (Difco)	Direct plate count	Henning et al. (2004)	
Listeria sp. Count (LC)	Oxford (Oxorid)	Cultural method	J	
Yeast & mould count (Y & M)	Potato dextrose agar (PDA)	Direct plate count	Frank et al. (2004)	

#### Table (1). Microbiological tests, media and methods for Kishk examination

#### Identification of LAB isolates from Kishk

Phenotypic characteristics including Gram strain, catalase enzyme and shape were done on one hundred isolates previously picked and purified from plates of MRS agar according to Harrigan & McCance (1986). Gram positive, catalase negative cocci and rods were further identified to species level adopt biotype method using API 20 and API 50 CHL strips respectively according to manufacturer instructions (Bio Merieux-France).

#### Physicochemical analysis

Analysis for moisture, protein, fat, ash and crude fibre were performed for forty Kishk samples procured from local market at four provinces located in Upper Egypt (Sa'eed) according to AOAC (2000). Carbohydrates as nitrogen free extract (NFE) were determined from the equation:

$$NFE = TS - (P + F + A)$$
(1)

Where TS, (Total Solids); P, (Protein); F, (Fat); & A, (Ash).

Value of energy (VE as Cal/ 100 g) was calculated from the equation:

$$VE = (Protein X 4.0) + (Fat X 9.0) + (Carbohydrate X 4.0)$$
 (2)

Nitrogen content was determined adopt Kjeldahl method. The obtained figure was multiplied by a factor of 6.25 to calculate the protein content of the Kishk samples. pH of Kishk was measured according to the method described by Tamime et al. (1999). Briefly, grind Kishk sample, rehydrate with water at a ratio of 1:2 (w/v) and pH was measured using electronic pH meter (ADWA 8000, Hungary).

#### Minerals content

Determination of Phosphorous (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn), Iron (Fe), Zinc (Zn) and Cupper (Cu) in Kishk was carried out according to Chapman and Pratt (1978). P was measured using spectrophotometer (Perkin

Elmer) UV/VIS spectrophotometer Lambda 2 while, atomic absorption spectrophotometer Perkin Elmer 1100B was used to measure the remaining aforementioned minerals.

#### Statistical Analysis

Data were expressed as the mean values  $\pm$ standard error (SE) for three replicates and statistically analyzed by performing analysis of variance technique using the Statistical Analysis System (SAS, 1996). Differences among means were compared using Duncan's Multiple Rang Test (Duncan, 1955) at significant level 95% (p≤0.05).

## **RESULTS AND DISCUSSION**

#### Microbiological counts

Table (2) reveals the mean total viable counts (TVC) prevails in Kishk samples from Assiut (A), Beni-Swif (B), Fayoum (F) and Giza (G) provinces in Upper Egypt. Figures are means $\pm$ SE of 10 samples from each province reported as  $\log_{10}$  cfu/g. Log TVC were  $3.35\pm0.10$ ,  $3.37\pm0.12$ ,  $2.96\pm0.49$  and  $2.36\pm0.24$  for A, B, F and G provinces respectively. No significant difference (p $\ge0.05$ ) between A & B provinces, while a significant difference was shown between both of them and F & G provinces respectively. The frequency distribution is the same, i.e. (100%) in all Kishk samples. Log lactic acid bacteria counts (LAB) were  $3.58\pm0.12$ ,  $3.60\pm0.16$ ,  $4.35\pm0.41$  and  $1.24\pm0.62$  for A, B, F & G provinces, respectively. Differences between LAB counts are coincide with those for TVC as well as their frequency distribution. Enterococcus count (EC) figures were  $2.88\pm0.33$ ,  $3.22\pm0.38$ ,  $0.61\pm0.10$  and  $0.23\pm0.32 \log_{10}$  for A, B, F & G provinces in this order. A significant difference in log counts could be observed between the four provinces as shown by Table (2). Also the frequency distribution for A, B & F provinces was 100%, while it reached only 50% for G province.

Positive incidence of coliform expressed as log counts were 1.01±0.18 and 0.21±0.25 for A & G provinces, respectively. On the other hand, coliform counts (CC) could not be detected in Kishk from B & F provinces, respectively. The frequency distribution of (CC) was 50% in province (A) and 80% in province (G). Coagulase +ve Staphylococcus aureus were not detected in any Kishk sample from the A, B, F & G provinces. This was verified by adding few drops of bromo thymol blue indicator to plate and looking for a yellow halo around the colonies then the plate is filled by 5 ml of saturated solution of ammonium sulphate and incubated for 15 min to observe the hydrolysis of gelatin. Colonies showed orange to golden color, mannitol +ve and clearing zone around the colony are potentially coagulase +ve Staphylococcus aureus. Referring to Table (2), no Listeria sp. could be detected under the condition of that experiment. However, for Y&M count, the log<sub>10</sub> were 1.62 + 0.32, 1.50+0.28, 1.75+0.11 and 2.01+ 0.09 for A, B, F and G provinces respectively. No significant difference was observed between Y&M counts for A&B provinces. Contrary was the situation between A, B, F and corresponding counts in G province. The frequency distribution was 50% for all provinces where it means that only 5+ve samples out of 10 samples examined were detected. It is clearly appeared from Table (2) that SK is safe for human consumption because it is free from pathogens pursued in the current study. This could be explained based on the lactic acid fermentation during SK preparation which minimize the pH, production of antimicrobials by LAB, low a<sub>w</sub> and non hygroscopic property of the product. Yeast & mould detected in SK samples could be attributed to aerial contamination during processing and handling or/ and from laban zeer, belila (the main ingredients in (SK).

## Identification of LAB from Kishk samples

The identified LAB isolated from Kishk samples are listed in Table (3). The LAB species were identified as *Lactococcus lactis* subsp. *Lactis, Lactococcus lactis* subsp.*cremoris, Leuconostoc lactis, Enterococcus faecium, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus casei and Lactobacillus plandarum. En. faecium* identified in the current

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investigation could be supported by the finding reported by Attia & Khattab (1985). While we had identified species of bacteria belong to three different LAB genera namely *Lactococcus, Leuconostoc and Lactobacillus*. Awad et al. (2013) identified ten species all were from the genus *Lactobacillus*. Laban zeer as a fermented buttermilk is expected to be overloaded with a wide array of LAB. This expectation was based upon the results reported by Saleh (2013).

	А	A B		F			G	
	log <sub>10</sub> cfu/g <sup>*</sup>	$\mathrm{FD}^{**}$	log <sub>10</sub> cfu/g	FD	log <sub>10</sub> cfu/g	FD	log <sub>10</sub> cfu/g	FD
Total viable count (TVC)	3.35±0.10 <sup>a</sup>	10/10	3.37±0.12 <sup>a</sup>	10/10	$2.96 \pm 0.49^{b}$	10/10	2.36±0.24 <sup>c</sup>	10/10
Lactic acid bacteria count (LAB)	3.58±0.12 <sup>a</sup>	10/10	3.60±0.16 <sup>a</sup>	10/10	4.35±0.41 <sup>b</sup>	10/10	1.24±0.62 <sup>c</sup>	10/10
<i>Enterococcus</i> count (EC)	2.88±0.33 <sup>a</sup>	10/10	$3.22 \pm 0.38^{b}$	10/10	$0.61{\pm}0.10^{ab}$	5/10	6.23±0.32 <sup>c</sup>	5/10
Coliform count (CC)	1.01±0.18 <sup>a</sup>	5/10	ND	0/10	ND	0/10	0.21±0.25 <sup>b</sup>	8/10
Coagulase +ve Staphylococcus aureus count (CPSC)	ND	0/10	ND	0/10	ND	0/10	ND	0/10
<i>Listeria</i> sp. Count (LC)	ND	0/10	ND	0/10	ND	0/10	ND	0/10
Yeast & mold count (Y & M)	1.62±0.32 <sup>a</sup>	5/10	1.50±0.28 <sup>a</sup>	5/10	$1.75 \pm 0.11^{ab}$	5/10	2.01±0.09 <sup>c</sup>	5/10
A (Assiut), B (Beni-Swif), F (Fayoum), G (Giza) provinces $* \log_{10} cfu/g = \log_{10} colony$ forming unit/g								

Table (2). Average microbial counts (log<sub>10</sub> cfu/g) and its frequency distribution in Kishk

\*\* *FD* = *Frequency distribution of positive samples* 

*ND* = *Not detected* 

Means within the same line followed by the same superscripted letter(s) are not significantly different  $(p \ge 0.05)$ 

Species identification	N. of isolate		
Lactococcus lactis subsp. lactis	18		
Lactococcus lactis subsp.cremoris	7		
Leuconostoc lactis	3		
Enterococcus faecium	32		
Lactobacillus delbrueckii subsp. bulgaricus	20		
Lactobacillus casei	12		
Lactobacillus plandarum	8		

## Table (3). Identification of LAB isolated from Kishk using API system

## Physicochemical analysis of Kishk samples

Data in Table (4) showed that the highest values of moisture content were recorded for Beni Suef (B) and Giza (G) Kishk samples. Meanwhile, there is no significant difference in between the two figures determined for both provinces; however, there is a significant difference ( $p \le 0.05$ ) between them and Assiut (A) Kishk sample. With respect to protein and fat content for samples of province (A) it could be observed that there is a significant increase in these two items ( $p \le 0.05$ ) compared to other data for provinces B, F, and G respectively. The lowest values of protein and fat contents being 11.43%, 11.60% & 3.43, 3.21% were recorded for (G)

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and (B) Kishk samples respectively. The highest value of ash content were observed in case of (G) and (B) Kishk samples (8.473, 8.200%, respectively), while the lowest values were found in (F) and (A) Kishk sample (4.843 and 3.383%, respectively. However, a significant difference  $(p \le 0.05)$  between ash content in (F) and (A) could apparently observed. There is no significant difference between (B), (F) and (G) Kishk samples with respect to their fiber contents. In (A) Kishk samples, a distinct finding was achieved with regard to its low fiber content with a value of (1.177%). Carbohydrate content fluctuated in such traditional dairy foods of the four provinces. (F) Kishk samples has a highest value followed by (B) and (G) samples in a descended order. Contrary, (A) Kishk samples had lowest values. Similar results have been reported by Tamime & O'Connor (1995), they reported that there are major differences in the chemical composition of commercial samples of Kishk made in different Middle Eastern countries: 3.9-13.0% moisture, 8.9-54.5% protein, 1.6-19.9% fat, 31.0-65.7% carbohydrates, 0.5-2.5% fiber and 3.8-9.5% ash. Morcos et al. (1973) reported an average protein content of 21.6 g/100 g<sup>-1</sup> of the Egyptian Kishk samples analyzed. Fat values were similar to fat contents of Kishk made in different countries of the Middle East. The variation in the fat contents of the Kishk samples could be attributed to the efficiency of fat separation from the milk before the production of yoghurt, to the efficiency of recovery of the butter granules from the churned fermented milk, or to the ratio of fermented milk/cereal mixture used (Tamime & O'Connor, 1995). Value of energy recorded a significant differences, a high value for (A) Kishk sample 380.76 % followed by (F) 371.473%, while there is no significant difference ( $P \ge 0.05$ ) between (B) and (G) samples. These variations in these levels could be attributed to many factors such as: (a) the preparation of Kishk and the added ingredients used; (b) the time used for drying the fermented milk/burghol mixture in the sun; (c) the type or combinations of milk used for fermentation; (d) the efficiency of fat separation after churning the fermented milk; and (e) the amount of salt that is added, (Tamime et al., 1999).

		Chemical composition*							
Kishk Sample	Moisture	Total solid	Protein	Fat	Ash	Fiber	Total carbohydrates (NFE)**	Value of energy Cal/100g***	pH value
Assiut	6.82 <sup>b</sup>	93.18 <sup>a</sup>	18.84 <sup>a</sup>	4.85 <sup>a</sup>	3.38 <sup>c</sup>	1.18 <sup>b</sup>	65.43 <sup>c</sup>	380.76 <sup>a</sup>	2 75
(A)	$\pm 0.08$	$\pm 0.08$	$\pm 0.46$	$\pm 0.25$	±0.09	±0.09	$\pm 0.42$	$\pm 1.57$	5.75
Beni Swif (B)	7.24 <sup>a</sup> ±0.003	92.69 <sup>c</sup> ±0.07	11.60 <sup>b</sup> ±0.22	3.21 <sup>b</sup> ±0.19	8.20 <sup>a</sup> ±0.03	1.48 <sup>a</sup> ±0.03	69.83 <sup>b</sup> ±0.21	354.03 <sup>c</sup> ±1.08	3.90
Fayou	$7.02^{ab}$	92 98 <sup>ab</sup>	11 87 <sup>b</sup>	3 51 <sup>b</sup>	4 84 <sup>b</sup>	1 53 <sup>a</sup>	73 09 <sup>a</sup>	371 47 <sup>b</sup>	
m (F)	±0.09	±0.09	±0.30	±0.18	$\pm 0.17$	±0.06	±0.24	±2.67	3.96
Giza (G)	7.23 <sup>a</sup> ±0.07	92.77b <sup>c</sup> ±0.07	11.43 <sup>b</sup> ±0.18	3.43 <sup>b</sup> ±0.12	8.47 <sup>a</sup> ±0.11	1.53 <sup>a</sup> ±0.05	69.43 <sup>ь</sup> ±0.39	354.32 <sup>c</sup> ±0.85	4.20

#### Table (4). Physicochemical composition of Kishk samples

<sup>#</sup> Means of three replicates ± standard error.

*Means within the same column followed by the same superscripted letter(s) are not significantly different at* ( $P \ge 0.05$ ).

\*\* NFE: Nitrogen Free Extract. \*\*\* Value of energy as Cal/ 100g

Egyptian provinces. Contrary to our pH figures, other investigators stated that pH of kashk (Iranian Kishk- like) revealed pH of 4.31 (Mashak et al., 2014). Such variation could be

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attributed to the variation in method of processing, the microorganisms responsible for fermentation and the pH of the ingredients used.

#### **Minerals** content

Worthy mention, El-Nawawy et al. (2012) had determined six mineral in commercial Egyptian Kishk namely, K, Ca, Mg, Fe, Na and Zn. Their data were 179.02, 121.46, 129.72, 2, 339.15 and 2.79 mg /100g sample for the aforementioned elements respectively. Element K in our results (See Table 5) is higher for A, B, & F provinces and was the highest in G province. The same trend was observed for element Ca except with the G one. The reason that G Kishk showed such high concentration may be attributed to more than one factor such as the kind of the soil, the variety of the wheat cultivated in Giza province or other unknown reason which necessitate the need to specify another point of research in the future. Colleagues with the nutrition or crop production in the Egyptian scientific community are invited to participate in a collaborative work in this interested discipline.

			Kishk samples						
Mineral		Assiut	Beni Swif	Fayoum	Giza				
			(A)	(B)	(F)	(G)			
1.	Phosphors	Р	321	239	317	255			
2.	Potassium	Κ	475	560	470	1540			
3.	Calcium	Ca	255	180	83	940			
4.	Magnesium	Mg	85	85	87	85			
5.	Sodium	Na	181	338	231	323			
6.	Iron	Fe	315	285	297	497			
7.	Manganese	Mn	31	20	29	25			
8.	Zinc	Zn	163	161	167	166			
9.	Cupper	Cu	10	9	30	9.9			

Table (5). Mineral content of Kishk samples

Elements from 1-5 are (mg per 100 g); sample from 6-9 are (ppm).

## CONCLUSION

Kishk has good microbiological and physicochemical qualities. Milk fermentation resulting in better absorption of calcium ions important for both young and elder peoples. Kishk has a long shelf life at ambient high temperature prevail at Sa'eed provinces without refrigeration. The impact of this study was to provide information to decision makers at policy- level and those involved in development programmers about technical support for initiation a low coast small enterprises that can contribute to small scale farmer's income. Additionally, more research is needed toward improving the traditional method of processing Kishk using a balanced probiotic starter cultures. Research on Kishk structure and production on an industry scale deserve future research efforts.

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الخواص المكروبيولوجيه والكيميائية للكشك المصرى الصعيدى

سالم عبد الغنى حسن ، وفاء كامل بهجات و محمد توفيق فوَّاد قسم الألبان ـ شعبة بحوث الصناعات الغذائية والتغذية ـ المركز القومي للبحوث ـ الدقي ـ الجيزة ـ جمهورية مصر العربية

الهدف من هذه الدراسة تقييم الجودة الميكروبيولوجية والخواص الفيزيوكيميائية ومحتوى المعادن فى الكشك الصعيدى لهذا جمعت اربعون عينة من الكشك من اربع محافظات بصعيد مصر هى أسيوط ،بنى سويف، الجيزة، الفيوم بواقع عشر عينات من كل محافظة .

تم تقدير المحتوى الكلى من البكتريا، بكتريا حامض اللكتيك ومجموعة المكورات المعوية فى الكشك على أساس انها تتواجد بصورة طبيعية فى هذا المنتج الغذائى. كما قدرت أعداد كل من بكتريا مجموعة القولون، الليستريا، المكورات العنقودية، الخميرة والفطر. كما تم عزل وإنتقاء مائة مستعمرة من بكتريا حامض اللكتيك وتصنيفها بالطرق المظهرية والحيوية إلى مستوى النوع. بالاضافة إلى ذلك تم تقدير الخواص الفيزيوكيميائية لعينات الكشك متمثلة فى تقدير الرطوبة، الجوامد الكلية، البروتين، الدهن، الرماد، الألياف الكلية والكربوهيدرات الكلية ،ورقم الاس الهيدروجيني كذلك تم تقدير والكشف عن وجود تسعة عناصر معدنية فى الكشك هي ( الفوسفور، البوتاسيوم، الكالسيوم، الماغنسيوم، الصوديوم، الحديد، المنجنيز، الزنك والنحاس) بنسب متفاوتة.

وتشير مجمل النتائج إلى أن العينات تحت الدراسة كانت خالية من البكتريا المرضية التي تنتقل عن طريق الغذاء وأن المنتج ذو قيمة غذائية عالية وتنوفر فيه كثير من العناصر المعدنية. بالإضافة لذلك فقد تم عزل وتصنيف سلالات مميزة من بكتريا حامض اللاكتيك يمكن استخدامها كبادئات مساعدة في تصنيع منتجات لبنية ذات خصائص وظيفية فريدة.

**الكلمات الدالة:** الكشك الصعيدي المصري، بكتريا حمض اللاكتيك، الخواص الفيزيوكيميائية