

## Kishk – a dried fermented milk/cereal mixture. 4. Microbiological quality

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**Abstract** — The microflora of 25 samples of Kishk were determined. Pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Listeria* sp., *Campylobacter* sp., *Yersinia* sp. and *Brucella* sp. were not recovered from any of the samples at the levels tested. Enterotoxins of staphylococci, bacilli and clostridia also were not detected. *Bacillus cereus*, aerobic spores, total viable count and contaminants were recovered in appreciable numbers, and some *B. cereus* strains that may produce toxins could be the major bacteriological risk associated with this product. Coliforms, faecal enterococci and *Clostridium perfringens* were only recovered from samples 7 and 1, respectively. However, yeasts and moulds were recovered intermittently from some of the Kishk samples, and the possible high count of yeast ( $8.5 \times 10^5$  cfu·g<sup>-1</sup>) could be attributed to the possible use of 'artisan'-type starter culture containing lactose fermenting yeast. The lactic acid bacteria counts were up to  $3.0 \times 10^3$  cfu·g<sup>-1</sup> and  $1.2 \times 10^6$  cfu·g<sup>-1</sup> for lactococci and lactobacilli, respectively. The overall inherent characteristics of Kishk (0.4 water activity [A<sub>w</sub>], 3.8 pH, high in salt and contains a wide range of organic acids) contributes towards the microbiological safety of the product. © Inra/Elsevier, Paris.

### Kishk / microbial count and toxin / water activity

**Résumé** — Le kishk – un mélange lait/céréales fermenté et séché. 4. Qualité microbiologique. La microflore de 25 échantillons de kishk a été déterminée. Les bactéries pathogènes telles que *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Listeria* sp., *Campylobacter* sp., *Yersinia* sp. et *Brucella* sp. n'étaient pas présentes dans les échantillons aux niveaux testés. Les entérotoxines de staphylocoques, de bacilles ou de clostridies n'ont pas non plus été détectées. Des quantités appréciables de *Bacillus cereus*, spores aérobies, flore totale et contaminants étaient dénombrées ; certaines souches de *B. cereus* qui peuvent produire des toxines constitueraient le principal risque bactériologique associé à ce produit. Des bactéries coliformes, entérocoques fécaux et *Clostridium*

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*perfringens* ont été trouvés seulement dans les échantillons numéros 1 et 7. Cependant, des levures et des moisissures étaient retrouvées par intermittence dans quelques échantillons de kishk, et la population élevée de levures ( $8,5 \times 10^5$  ufc·g<sup>-1</sup>) pourrait être attribuée à l'utilisation d'un levain « artisanal » contenant des levures fermentant le lactose. Les dénombrements des bactéries lactiques atteignaient  $3,0 \times 10^3$  ufc·g<sup>-1</sup> et  $1,2 \times 10^6$  ufc·g<sup>-1</sup> respectivement pour les lactocoques et les lactobacilles. Les caractéristiques physico-chimiques du kishk (Aw de 0,4 ; pH de 3,8 ; forte teneur en sel ; forte variété d'acides organiques) contribuent à assurer la sécurité microbiologique de ce produit. © Inra/Elsevier, Paris.

## kishk / dénombrement microbien / toxine microbienne / activité de l'eau

### 1. INTRODUCTION

The traditional method for the manufacture of Kishk is very basic [24], and the microbiological quality of the product is mainly governed by factors such as: 1) the method used to ferment the milk; 2) the hygienic conditions practised during the manufacturing stages including whether or not the milk was heat treated (e.g. boiled) before the fermentation stage; and 3) the drying stage in the open air in the sun. Three possible types of lactic fermentation may be distinguished during the manufacture of Kishk: first, unknown composition of the starter culture where the indigenous microflora is used to ferment the unheated milk; second, 'artisan' starter culture is normally used frequently by seeding the milk with yoghurt or another type of fermented milk from the previous day's batch, and hence, the starter composition may be variable; and third, selected lactic starter cultures obtained from commercial sources of known composition.

It is evident that acidification of milk can extend the shelf-life of the manufactured products, and limited data are available on the microbiological quality of Kishk. Nevertheless, the low moisture content (< 10 %), acidic nature of the product (~3.8 pH) and the addition of salt during manufacture (~2.8 g·100 g<sup>-1</sup> NaCl in the dried product) may suggest the microbiological safety of Kishk [25].

Faecal enterococci count at a level of  $3.4 \times 10^2$  colony forming units (cfu)·g<sup>-1</sup> was reported by Atia and Khattab [2] in only one of eight Egyptian Kishk samples tested. However, the groups of undesirable microorganisms, which were found in different commercial samples of Kishk, were mainly spore-formers (i.e. *Bacillus* spp.), yeasts and moulds [24]. In Iran, the death of two people, who had clinical symptoms of botulism food poisoning, was associated with the consumption of Kishk, and Haydarynia [9] reported the growth, survival and production of toxin of *Clostridium botulinum* in laboratory-made Kishk.

In previous studies the chemical and nutritional properties of Kishk were detailed [25–27]. The objective of this study was to investigate the microbiological evaluation of 25 different samples of Lebanese Kishk.

### 2. MATERIALS AND METHODS

#### 2.1. Kishk samples

Twenty-five samples of commercial Kishk (~1 kg each) were obtained from different retail outlets in Lebanon [25].

#### 2.2. Analytical methods

##### 2.2.1. Water activity ( $A_w$ ) and pH

The water activity in the Kishk samples was determined according to the method described

by O'Brian [21]. All measurements were made with an  $A_w$  Portimeter Dewpoint Meter DP 383R (Portimeter Ltd., Marlow, UK) placed in a warm room at a nominal temperature of 25 °C. As the Kishk products had low  $A_w$ , equilibration of the chamber for 24 h was employed. A standard solution of NaCl was checked weekly as a control.

The pH in the Kishk samples was measured according to the method described by Tamime et al. [25].

### 2.2.2. Microbiological analysis

The Kishk samples were transported to Scotland by air at ambient temperature and stored at ~5 °C on arrival for around 6 months. A wide range of microbiological tests including toxin determinations were carried out, and *table 1* summarises such analysis, including the methods used. The preparation of each sample and dilutions for microbiological examination was carried out according to the method described by IDF [20]. Each Kishk sample (10 g) was homogenised in 90 mL of sterile 2% sodium citrate solution at ~7 °C for 15 s in a Colworth Stomacher 400 model BA 6021 (A.J. Seward Medical, London, UK). Serial dilutions were made in 1/4 strength sterile Ringer's solution and plated in duplicate on specific agar, and the average of the determinations was expressed as cfu·g<sup>-1</sup>.

### 2.3. Statistical analysis

The data were analysed using graphical exploratory data analysis techniques available in Minitab 10.2 (copyright Minitab, Inc., 1994).

## 3. RESULTS AND DISCUSSION

### 3.1. Chemical and nutritional composition of Kishk samples

The average chemical composition (protein, fat, carbohydrates, ash, organic acids and fatty acids) and micronutrients (mineral, selenium, amino acids and vitamins) including  $\beta$ -glucan, fibre, phytic acids contents and profiling of the proteins of 25 samples of Lebanese Kishk have been reported by Tamime et al. [25–27].

**Table I.** Methods used for the microbiological analysis of Kishk<sup>a</sup>.

**Tableau I.** Méthodes utilisées pour l'analyse microbiologique du kishk<sup>a</sup>.

Microorganism/ test method	Reference
Total viable count <sup>b</sup>	[17]
Total microbial contaminants <sup>c</sup>	[16]
Psychrotrophic count	[18]
Coliforms (MPN) <sup>d</sup>	[11]
<i>Staphylococcus aureus</i>	[14]
Lactic acid bacteria <sup>e</sup>	[12, 19]
Yeasts and moulds <sup>f</sup>	[15]
<i>Listeria monocytogenes</i>	[13]
<i>Salmonella</i> spp.	[10]
<i>Escherichia coli</i> (MPN)	}
<i>Bacillus cereus</i>	}
<i>Clostridium perfringens</i>	}
<i>Enterococcus faecalis</i> (MPN)	} <sup>g</sup> [4]
<i>Aeromonas hydrophila</i>	}
<i>Pseudomonas</i> spp.	}
<i>Campylobacter</i> spp.	}
Clostridia (MPN)	[7]
Aerobic spore former count	[8]
<i>Yersinia</i> spp.	[22, 23]
<i>Brucella</i> spp.	[6]

<sup>a</sup> Most of the tests used in this study were performed on spiral plate technique as opposed to spread or pour plate; <sup>b</sup> total count of bacteria, yeasts and moulds; <sup>c</sup> non-lactic acid bacteria, yeasts and moulds; <sup>d</sup> MPN: most probable number; <sup>e</sup> thermophilic lactic acid bacteria mainly to enumerate yoghurt starter cultures; <sup>f</sup> oxytetracycline was replaced by chloramphenicol; <sup>g</sup> test methods were described by the Oxoid Manual including the enterotoxin detection of *S. aureus*, *B. cereus* and *C. perfringens*.

<sup>a</sup> La plupart des tests dans cette étude ont été réalisés par ensemencement spiral plutôt que par étalement ou ensemencement dans la masse. <sup>b</sup> Dénombrement total des bactéries, levures et moisissures. <sup>c</sup> Bactéries non-lactiques, levures et moisissures. <sup>d</sup> MPN – nombre le plus probable. <sup>e</sup> Bactéries lactiques thermophiles (principalement pour dénombrier les levains du yaourt). <sup>f</sup> L'oxytétracycline a été remplacée par du chloramphenicol. <sup>g</sup> Les méthodes sont décrites dans le manuel Oxoid avec la détection des enterotoxines de *S. aureus*, *B. cereus* et *C. perfringens*.

### 3.2. Water activity ( $A_w$ ) and pH

The residual microbial activity in food products is dependent on a multitude of factors such as availability of nutrients, storage conditions, acidity, salt level and/or water activity. The  $A_w$  readings of 25 samples of the Kishk ranged between 0.34 and 0.43 (table II), which are proving to be rather low to support the growth of microorganisms that not even the most xerophilic mould could grow.

The salt, pH and certain organic acids contents of these Kishk samples have been also included in table II for comparative purposes where such data may provide the relevant information that may influence the survival of certain microorganisms in the dried product. For example, the  $pK_a$  of lactic < acetic < propionic acids [5], and the undissociated acid is more active as an antimicrobial to inhibit the growth of pathogens and other undesirable microorganisms [1].

### 3.3. Microbiological quality

The microorganisms that have been detected in the Kishk samples are shown in table III. The total viable count (TVC)

in 20 samples of Kishk ranged between  $3.1 \times 10^2$  and  $1.1 \times 10^6$  cfu.g<sup>-1</sup>. No TVC was recovered from Kishk samples 21, 23 and 24, suggesting that the fermented milk/cereal mixture was subjected to heat treatment before drying; however, counts for samples 4 and 5 could not be carried out due to the presence of spreading colonies, probably *Bacillus* sp. Similar pattern of counts were observed when the total contaminant count (i.e.  $2.8 \times 10^2$  to  $1.3 \times 10^6$  cfu.g<sup>-1</sup>) was performed (table III), including Kishk samples 4, 5, 21, 23 and 24 (for sample identification, refer to [25]). Similar total counts of commercial and laboratory-made Kishk have been reported elsewhere [24]. However, the low microbial contaminants count ranged between < 10 and  $4.7 \times 10^2$  cfu.g<sup>-1</sup> for Kishk samples 1, 2, 7, 9, 10, 21, 23 and 24, whilst highest counts ( $1.3 \times 10^5$  to  $1.3 \times 10^6$ ) were recovered from samples 3, 12, 13, 16 and 17. The high microbial contamination in these Kishk samples reflect the poor sanitary conditions during the manufacturing stages or post-production contamination, and interestingly four of these products were made by non-dairy organisations.

The presence of coliforms were only detected in Kishk samples 3 and 13 which yielded 24 and 43 MPN (most probable num-

**Table II.** Measurements of available water, pH, salt and certain organic acid contents of different samples of Lebanese Kishk.

**Tableau II.** Détermination de l'eau disponible, du pH, du sel et de certains acides organiques dans différents échantillons de kishk libanais.

Component	Minimum	Maximum	Mean
Water activity ( $A_w$ )	0.34	0.43	0.40
Salt (g-100 g <sup>-1</sup> ) <sup>a</sup>	1.0	4.5	2.9
pH <sup>a</sup>	3.63	4.12	3.77
Organic acids (mg.g <sup>-1</sup> ) <sup>a</sup>			
Lactic	18.75	43.87	32.47
Acetic	0.40	0.86	0.59
Propionic	0.92	7.45	3.55

<sup>a</sup> Data compiled from [25].

<sup>a</sup> Données issues de Tamime et al. [25].

**Table III.** Microbiological quality of different Kishk samples.**Tableau III.** Qualité microbiologique de différents échantillons de kishk.

Sample number <sup>a</sup>	Colony forming units (cfu)-g <sup>-1</sup>						Most probable number	
	Total count	Contaminants	Aerobic spores	<i>B. cereus</i>	Yeasts	Moulds	Coliforms	Faecal enterococci
1	$4.2 \times 10^4$	$4.7 \times 10^2$	< 10	< 10	< 10	< 10	< 0.3	< 0.3
2	$3.7 \times 10^4$	$2.8 \times 10^2$	< 10	< 10	$8.0 \times 10^2$	$6.0 \times 10^2$	< 0.3	< 0.3
3	$1.0 \times 10^6$	$1.0 \times 10^6$	$5.0 \times 10^3$	$6.0 \times 10^2$	$8.5 \times 10^5$	< 10	24	240
4	sc <sup>b</sup>	sc	$1.4 \times 10^6$	$1.3 \times 10^5$	< 10	< 10	< 0.3	< 0.3
5	sc	sc	$1.4 \times 10^6$	$7.8 \times 10^4$	< 10	< 10	< 0.3	< 0.3
6	$4.2 \times 10^4$	$2.8 \times 10^4$	$3.4 \times 10^3$	$1.5 \times 10^3$	$3.0 \times 10^3$	< 10	< 0.3	240
7	$6.0 \times 10^2$	$3.7 \times 10^2$	< 10	< 10	< 10	< 10	< 0.3	< 0.3
8	$1.8 \times 10^4$	$8.0 \times 10^4$	< 10	< 10	$7.0 \times 10^3$	< 10	< 0.3	9
9	$3.0 \times 10^2$	$2.8 \times 10^2$	< 10	< 10	$1.0 \times 10^3$	$1.6 \times 10^2$	< 0.3	< 0.3
10	$3.0 \times 10^2$	$3.4 \times 10^2$	$1.4 \times 10^3$	< 10	< 10	< 10	< 0.3	< 0.3
11	$9.5 \times 10^3$	$4.6 \times 10^3$	$6.1 \times 10^2$	< 10	$4.0 \times 10^3$	$1.0 \times 10^2$	< 0.3	24
12	$1.1 \times 10^6$	$1.0 \times 10^6$	$9.0 \times 10^5$	< 10	< 10	< 10	< 0.3	< 0.3
13	$9.2 \times 10^5$	$1.2 \times 10^6$	$1.3 \times 10^6$	$2.4 \times 10^4$	$2.2 \times 10^3$	< 10	43	93
14	$1.4 \times 10^3$	$2.2 \times 10^3$	$2.5 \times 10^3$	< 10	< 10	< 10	< 0.3	< 0.3
15	$1.1 \times 10^6$	$9.0 \times 10^5$	$4.1 \times 10^5$	< 10	< 10	< 10	< 0.3	< 0.3
16	$9.4 \times 10^4$	$1.3 \times 10^5$	$5.4 \times 10^4$	< 10	< 10	< 10	< 0.3	< 0.3
17	$1.3 \times 10^4$	$1.3 \times 10^6$	$1.2 \times 10^6$	$5.6 \times 10^4$	< 10	< 10	< 0.3	< 0.3
18	$3.2 \times 10^3$	$2.2 \times 10^3$	$6.4 \times 10^3$	< 10	< 10	< 10	< 0.3	< 0.3
19	$3.7 \times 10^3$	$3.0 \times 10^3$	$3.2 \times 10^3$	< 10	< 10	< 10	< 0.3	< 0.3
20	$1.7 \times 10^3$	$1.6 \times 10^3$	$1.8 \times 10^3$	< 10	< 10	< 10	< 0.3	240
21	< 10	< 10	< 10	< 10	< 10	< 10	< 0.3	< 0.3
22	$1.1 \times 10^4$	$9.0 \times 10^3$	$9.8 \times 10^3$	$2.4 \times 10^3$	< 10	< 10	< 0.3	43
23	< 10	< 10	< 10	< 10	< 10	< 10	< 0.3	< 0.3
24	< 10	< 10	< 10	< 10	< 10	< 10	< 0.3	< 0.3
25	$4.2 \times 10^3$	$5.4 \times 10^3$	$4.0 \times 10^3$	< 10	< 10	< 10	< 0.3	< 0.3

<sup>a</sup> For sample identification, refer to [25]. <sup>b</sup> spreading colonies, plates could not be counted.<sup>a</sup> Voir Tamime et al. [25] pour l'identification des échantillons. <sup>b</sup> Colonies filantes, les boîtes ne pouvaient pas être comptées.

ber), respectively. Faecal enterococci were only recovered from Kishk samples 3, 6, 8, 11, 13, 20 and 22 at low levels (table III). It may be significant to point out that samples 3 and 20 had relatively high pH (4.1) when compared with the rest of the Kishk samples (i.e.  $\text{pH} \leq 3.8$ ), and both samples had coliforms and faecal enterococci. The low counts of these indicator organisms in Kishk could be attributed to the die-off as a result of low  $A_w$  and low pH of the product (table II). In addition, the psychrotrophic count of  $1.0 \times 10^3 \text{ cfu}\cdot\text{g}^{-1}$  was only obtained in Kishk sample 3.

Aerobic spores were present in 17 samples of Kishk in numbers ranging from  $6.1 \times 10^2$  to  $1.43 \times 10^6 \text{ cfu}\cdot\text{g}^{-1}$  (table III). However, seven of the Kishk samples yielded *B. cereus* at levels ranging between  $6.0 \times 10^2$  and  $1.34 \times 10^5 \text{ cfu}\cdot\text{g}^{-1}$ . Similar counts have been reported by Atia and Khattab [2] on Egyptian Kishk. The presence of *B. cereus* in Kishk is not surprising as this microorganism is one of the common contaminants of milk, and is most likely to be present in the parboiled cracked cereal (Burghol) (Khaskheli and Tamime, unpublished data). *B. cereus* produce spores which are likely to survive in the Kishk. Some strains of *B. cereus* produce toxins which may cause food poisoning, and it could be considered the major bacteriological risk associated with this product; however, *B. cereus* toxin was not detected in any of the Kishk samples. Furthermore, *C. perfringens* was only recovered from Kishk sample 3 at a very low level ( $3.0 \times 10^{-1} \text{ cfu}\cdot\text{g}^{-1}$ ) which may not be a significant count bacteriologically, and no toxin was detected. The MPN of clostridia were 2.3 and 0.92 which were recovered from Kishk samples 3 and 7, respectively.

The yeasts and mould counts were recovered intermittently from the samples (table III). Moulds were only detected in Kishk samples 2, 9 and 11, and the counts were  $6.0 \times 10^2$ ,  $1.6 \times 10^2$  and  $1.0 \times 10^2 \text{ cfu}\cdot\text{g}^{-1}$ , respectively. The yeasts counts ranged between  $8.0 \times 10^2$  and  $8.54 \times 10^5 \text{ cfu}\cdot\text{g}^{-2}$ ,

and were recovered only in samples 2, 3, 6, 8, 11 and 13. Slightly lower yeasts and moulds counts have been found in Kishk [24], but the high yeast count could be attributed to the 'artisan' starter culture used which may contain lactose fermenting yeast [3].

With respect to lactococci and lactobacilli counts, spreading colonies appeared in the majority of the agar plates which made the LAB counts in the Kishk very difficult, and hence, the efficacy of the final count may be inaccurate. However, some of the counts ranged between  $1.4 \times 10^2$  and  $3.4 \times 10^3 \text{ cfu}\cdot\text{g}^{-1}$  for *Lactococcus* sp. and  $1.3 \times 10^2$  and  $1.2 \times 10^6 \text{ cfu}\cdot\text{g}^{-1}$  for *Lactobacillus* sp. (counts are now shown).

In this survey, *Staphylococcus aureus*, *Escherichia coli*, *Aeromonas* sp., *Pseudomonas* sp. psychrotrophic count and *Brucella abortus* were not recovered from any of the Kishk samples at the level tested (i.e.  $10^{-1}$  dilution), and *Salmonella* sp., *Listeria* sp., *Campylobacter* sp. and *Yersinia* sp. were not recovered in 25 g of the product.

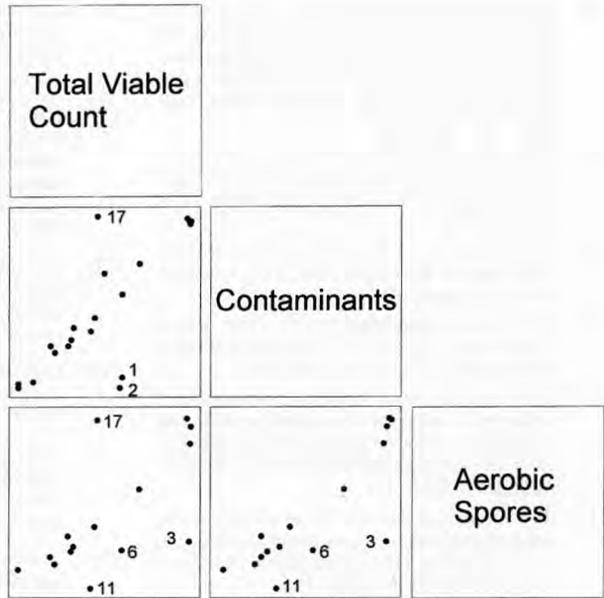
Figure 1 illustrates a matrix plot for TVC, contaminants count and aerobic spores count. When the labelled outlier Kishk samples were omitted from the calculations, all three variable microbiological counts were strongly correlated. The outlier samples did not have anything in common with each other nor were they distinguished from the other samples except when plotted. There were insufficient colony counts for the enumeration of the remaining microorganisms shown in table III to examine these counts in detail. However, when the microbial counts in figure 1 were related to variables (i.e. source, type of milk or packaging system; see table I in [25]), there did not appear to be any correlation between them.

#### 4. CONCLUSION

Given the conditions during the manufacture of Kishk, the overall indicator

**Figure 1.** Scatter plot of total viable count, contaminants count and aerobic spores count of Kishk samples.

**Figure 1.** Représentation de la dispersion des dénombrements en flore totale, contaminants et spores aérobies dans les échantillons de kishk.



microorganisms are only present in low numbers in the product and in microbiological terms, are relatively safe and stable. This could be attributed to a multitude of factors such as: low water activity, high salt and the presence of a wide range of organic acids and low pH. However, in present-day terminology these would be considered as 'hurdles', and it is interesting to observe how a traditional method of manufacture effectively mimics present-day food safety principles at least as far as preventing the growth of spores and *B. cereus* toxin. In addition, the intended use of Kishk is rehydrating with water followed directly by cooking to prevent the growth of undesirable microorganisms. There are indications in these microbiological results that the product quality is variable, with some samples showing noticeably more contamination than others. This is probably a reflection of the standards of hygiene applied during production and the quality of raw materials used, mainly the Burghol, and it indicates that with improved hygiene, the microbiological quality of the Kishk could be improved.

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