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STUDIES ON HYGIENIC STATUS OF KISHK PRODUCT

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ABSTRACT

This study was carried out for monitoring the bacteriological and mycological quality of Kishk. Thirty six random samples of Kishk were collected from different farmer producer and shops in Assiut city and some villages around it. All samples were subjected to microbiological examinations and mycotoxine investigation. *Enterococci* spp., Aerobic bacteria, Anaerobic bacteria and "Yeasts and molds" were isolated in 61.6, 100, 86.1 and 77.8 % of the examined Kishk samples, respectively, and with average total counts of 6.95 $x10^4$, 2.26 x 10^5, -- , and 3.9 $x10^4$, respectively. The presence of high contamination in Kishk samples reflects the poor sanitary conditions during the manufacturing stages or post production. *Aspergillus flavus, Aspergillus niger* and *Mucor* spp. were detected in 22.2, 16.7 and 8.3 % of the examined Kishk samples, respectively. Aflatoxin M_1 was found in 23.53 % of the samples. The concentration of aflatoxin M_1 in positive samples was 139 - 221 ng/Kg (average 149.8 ± 56.5 ng/Kg) by ELISA and 56 – 218 ng/Kg (average 135.3 ± 61.5 ng/Kg) by TLC. Kishk samples from Assiut city presented a high incidence of AFM_1 at levels below the limits established by Egyptian regulations. The general human exposure to AFM_1 by the consumption of contaminated kishk is probably non-significant in Egypt. However, the fact that AFM_1 is a potent hepatocarcinogen warrants concern about its occurrence in kishk. A high percentage of positive samples would be considered inappropriate for human consumption, when considering the tolerance limit adopted by the European Community. It could be concluded that, the product quality is variable, with some samples showing noticeably more contamination than others. This is probably a reflection of the standards of hygienic measures applied during production and the quality of raw materials used (fermented milk and crushed dried boiled wheat grains).

Key words: *Kishk, Fermented milk, Crushed wheat grains.*

INTRODUCTION

Kishk is one of the natural healthy food products, which have great taste and cultural values that are increasingly attractive to the Egyptian consumers. The traditional method for the manufacture of Kishk is very basic (composed mainly of crushed dried boiled wheat grains and acidified milk). 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 practiced during the manufacturing stages including whether the milk was heat treated (boiled) or not before the fermentation stage; and 3- the drying stage in the open air in sunny areas (Tamime and O'Conner, 1995).

Three possible types of milk fermentation may be distinguished during the manufacture of Kishk: first, is the use of 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 prolong the shelf-life of the manufactured products. Likewise, limited data are available on the microbiological quality of Kishk. Nevertheless, the low moisture content (10 %), acidic nature of the product (pH 3.8) and the addition of salt during manufacture (2.8 g NaCI / 100 g dried product) may be the cause of microbiological safety of Kishk (Tamime *et al.,* 1999).

It's reported that, *Lactobacilli* isolated from dairy products have shown a long history of safe use (WHO/FAO, 2001). They are used widely as starter

cultures in the food industry, e.g. fermented milk or meat products, alcoholic beverages, sourdough and silage (Carr *et al.,* 2002). Furthermore, cultures of various *Lactobacillus* strains have been developed for commercial use as probiotic bacteria.

The genus *Lactobacillus* has a long history of safe use, especially in the dairy industry, and plays a major role in the production of fermented milk products. Over the past few decades, an increased drive has existed for the isolation of novel *Lactobacillus* strains which termed probiotic exert a beneficial health effect when ingested by humans. Accordingly beneficial effects conferred by *lactobacilli* include inhibition of pathogenic organisms, such as *Salmonella*, *Shigella* and *Helicobacter* (Bernet-Camard *et al.,* 1997; Hudault *et al.,* 1997; Aiba *et al.,* 1998; Hammilton- Miller, 2003 and Sgouras *et al.,* 2004).

During the past two decades, probiotic (health promoting) microorganisms have been increasingly included in various types of food products, especially in fermented milks. Actually, a large population of probiotic bacteria is needed to carry out their benefit effect and to repel the harmful microorganisms causing disease. Indeed, some probiotic strains are rapidly killed by acid and bile, releasing active intracellular components as bacterial formylated peptides, peptidoglycan cell wall constituents and nucleotides (Ouwehand and Salminen, 1998; Salminen *et al.,* 1999 and De Vrese *et al.,* 2001). The technological application of probiotic organisms in fermented dairy products aims to combine the potential health benefits of the bacteria with their ability to grow in milk, resulting in a nutritionally healthy and desirable product for the consumers. Also, Gomez *et al.* (1997) evidenced a bacteriocin-like substance produced by a new strain of *Streptococcus spp*., inhibitory to Gram positive food-borne pathogens.

During the last fifteen years, the *Lactobacillus* genus has evolved and contains to date more than 80 species. They are present in raw milk and dairy products such as cheeses, yoghurts and fermented milks (Coeuret *et al.,* 2003*). Lactobacilli* comprise large and diverse group of Gram positive, non-spore forming, catalase negative rod bacteria, able to produce lactic acid as the main end-product of the fermentation of carbohydrates (Pelinescu *et al.,* 2009). They are considered as generally recognized as safe organisms and can be safely used as probiotics for medical and veterinary applications (Fuller, 1989).

Fermented milk present in Kishk product may be contaminated with *enterococci, coliforms* and *Escherichia coli*. *Mundt (1986)* demonstrated that the common presence of *E. faecalis* in many food products is not always related to direct faecal contamination. In 1992, the EU established a maximum level for the presence of *coliforms* and *Escherichia coli*, both considered as indicators of hygiene, while no limit was set for the enterococci (Anonymous, 1992). Furthermore, it has been shown that enterococci had little value as hygiene indicators in the industrial processing of foods (Bernet-Camard, *et al.,* 2001). Moreover, although *E. faecalis, E. faecium, and E. durans* are frequently isolated from human faeces, they are much less prevalent in livestock, such as pigs, cattle, and sheep *(*Franz *et al.,* 1999).

Fermented milk present in Kishk product may also contains aflatoxin M_1 (hepatocarcinogen) especially if the used milk obtained from animals fed on mycotoxine (aflatoxin B_1) contaminated ration (Park and Pohland, 1986). Aflatoxin M_1 (AFM_1) is the main metabolite produced by storage fungi of the genus *Aspergillus*, particularly *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius* (Moss, 1998). The occurrence of AFM_1 in milk and milk products is of public health concern. The *International Agency for Research on Cancer* (1993) has classified AFM_1 in group 2, a probable human carcinogen.

Many traditional foods are threatened as lifestyles are changed and local know-how is lost. Systematic investigation and registration of traditional foods can help to keep them on the menu, thus enabling future generation to experience and enjoy traditional local specialties. This work aimed to investigate the probiotic bacteria present in Kishk product and to evaluate its hygienic quality through the isolation and identification of some microorganisms that may contaminate it. Also, to detect the possible occurrence of aflatoxine M_1 in various locally manufactured Kishk.

MATERIALS and METHODS

1) Samples: collection, preparation and serial dilutions:

Thirty six random samples of Kishk collected in clean, dry and sterile containers from framer producing kishk at several villages and some shops in Assiut city. Samples transported directly to laboratory for investigations. Each sample was divided under complete hygienic measures with a clean, sterile knife into two parts; the first was prepared for microbiological examination. The preparation of each sample and dilutions was carried out according to the method described by A.P.H.A. (1992) and lDF (1992). 10 g of each Kishk sample was homogenized in 90 ml of sterile 2 % sodium citrate solution at 7º C for 15 s. 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. The other part was used for mycotoxins estimation.

2) Enumeration, isolation and identification of *Lactoabacilli* bacteria was carried out according to *De Man et al.* (1960) with the using of MRS agar and incubation at anaerobic conditions at 37°C for 48 hrs.

3) Enumeration, isolation and identification of *Enterococci* was carried out according to Deibel and Hartman (1982) using KF streptococcal agar and Azide dextrose broth.

4) Estimation of total bacterial count was carried out *according* to A.P.H.A. (1992) by using standard plate count agar.

5) Estimation of total yeasts and molds count was carried out according to Harrigan and MacCance (1976) by using malt extract agar contained 500 mg of chlortetracycline and chloramphenicol.

6) Detection of anaerobic bacteria using Stormy fermentation test according to Crückshank *et al.* (1969).

7) Detection of Aflatoxin M_1: Thirty four samples were randomly chosen for the estimation of the aflatoxin M_1. Fifty grams of each chosen sample were mashed, homogenized with organic solvents and extracted for estimation of the mycotoxins. The toxins were extracted from the kishk samples with a mixture of acetonitrile: phosphoric acid, 0.1M (10:1, V/V) and the extract was purified by liquid-liquid partition with isooctane to remove lipid material and with a mixture of aqueous sodium hydrogen carbonate and sodium chloride to separate acids from the neutral substances. Detection of Aflatoxin M_1 (AM_1) by using ELISA was carried out according to Thirumalla-Devi *et al.* (2002) and by using thin layer chromatography according to Egmond and Paulsch (1986) and Schuller and Egmond (1991) (Multimycotoxin analysis).

RESULTS

Physical examination of each Kishk sample was carried out by naked eye and revealed absence of any abnormal findings. Isolated microorganisms (% of positive samples and total count / g) and percentage of isolated fungi in the examined Kishk samples are shown in Tables 1 and 2 respectively. Aflatoxin M_1 (ng / Kg) in examined Kishk samples are shown in Table 3.

Table 1: Isolated microorganisms in the examined Kishk samples.

Isolated microorganisms	Examined samples (No.: 36)				
	Positive samples		Total counts / g		
	No.	%	Min.	Max.	Average
***Lactobacillus* spp.**	0	0	0	0	0
***Enterococci* spp.**	22	61.1	< 100	$1.58x\ 10^6$	$6.95x10^4$
Aerobic plate count	36	100	$5x10^3$	$2.20x10^6$	$2.26x10^5$
Detected anaerobic bacteria	31	86.1	-	-	-
Yeasts* and *fungi	28	77.8	< 100	$5.8x10^5$	$3.9x10^4$

Table 2: Types and percentage of isolated fungi in the examined Kishk samples.

Fungal species	Examined samples (No.: 36)	
	Positive samples	%
Aspergillus flavus	**8**	**22.2**
Aspergillus niger	**6**	**16.7**
***Mucor* spp.**	**3**	**8.3**

Table 3: Aflatoxin M_1 (ng / Kg) in the examined Kishk samples.

Number of examined samples	Estimation method	Positive samples		Concentration of Aflatoxin M_1 (ng/Kg) Mean ± SD
		Number	%	
34	TLC	8	23.53	135.3 ± 61.5
34	ELISA	8	23.53	149.8 ± 56.5

TLC: Thin layer chromatography ELISA: Enzyme linked immunosorbent assay.

DISCUSSION

Kishk made of acidified milk and crushed dried boiled wheat grains. It is rich in nutritive constituents and source for many vitamins, growth factors and other nutrients. Although, Kishk product has low moisture content (10 %), acidic nature (pH 3.8) and high percentage of salt (2.8 g NaCI / 100 g dried product) which may suggest its microbiological safety (Tamime *et al.,* 1999), it may be subjected to contamination with several organisms. In the present study *Enterococci* spp., Aerobic bacteria, Anaerobic bacteria and "Yeasts and Fungi" were isolated in 61.6, 100, 86.1 and 77.8 % of the examined Kishk samples, respectively, and with average total count of 6.95 $x10^4$, 2.26 x 10^5, - , and 3.9$x10^4$, respectively (Table 1). The presence of high contamination in Kishk samples reflects the poor sanitary conditions during the manufacturing stages or post production. Faecal *Enterococci* count at a level of 3.4 x 10^2 colony forming units (cfu/g) was reported by Atia and Khattab (1985) in only one of eight tested Egyptian Kishk samples. However, groups of undesirable microorganisms mainly spore-formers (i.e. *Bacillus spp*.), *yeasts and molds*, were found in different commercial samples of Kishk (Tamime and O'Connor, 1995). In Iran, the death of two people, who had clinical symptoms of botulism food poisoning, was found to be associated with the consumption of Kishk, the auther reported the growth, survivaI and production of toxin of *Clostridium botulinum* in laboratory-made Kishk (Haydarynia, 1990).

In recent years, the reports about *Enterococci* used as starter cultures or co-cultures (adjuncts) have increased considerably. Enterococci occur in soil, surface waters, and on plants, vegetables, and insects (Mundt, 1986). The resistance of *Enterococci* to pasteurization temperatures, and their adaptability to different substrates and growth conditions (low and high temperature, extreme pH, and salinity) implies that they can be found either in food products manufactured from raw materials (milk or meat) and in heat-treated food products. This means that these bacteria could withstand usual conditions of food production. In addition, they can contaminate finished products during food processing. Therefore, enterococci can become an important part of the fermented food microbiota, especially in fermented cheeses and meats. Moreover, although *E. faecalis, E. faecium,* and *E. durans* are frequently isolated from human faeces, they are much less prevalent in livestock, such as pigs, cattle, and sheep (Franz *et al.,* 1999).

Aspergillus flavus, Aspergillus niger and *Mucor* spp. were detected in 22.2, 16.7 and 8.3 % of the examined Kishk samples, respectively. Slightly lower yeasts and molds counts have been recorded in Kishk (Tamime and O'Connor, 1995). In previous study, high yeast count was fond in kishk samples and the author attributed this to the use of "artisan" starter culture which may contain lactose fermenting yeast (Baroudi and Collins, 1976).

Aflatoxin M_1 was found in 23.53 % of the examined Kishk samples. The concentration of aflatoxin M_1 in positive samples was 139 - 221 ng/Kg (average 149.8 ± 56.5 ng/Kg) by ELISA and 56 – 218 ng/Kg (average 135.3 ± 61.5 ng/Kg) by TLC. These findings revealed that the concentration of AFM_1 was relatively higher when estimated by ELISA than when estimated by TLC. Statistically there were insignificant variations between results obtained by the two methods used in the detection of aflatoxin M_1 in Kishk samples. These results indicated that the AFM_1, present in high levels in some samples and constitute a potential hazard for the consumers. It has been emphasized that consumption of aflatoxin M_1 probably constitute a much more serious public health problem. In many cases potential problems involve the possibility of carcinogens in human. Affected human may also predisposed to secondary infectious diseases because of the immune suppressive effect of the aflatoxins (Howard, 1983; Shan, 1991 and Oliveira and Germano, 1997).

The European Communities and Codex Alimentarius have fixed the limit to a maximum of 50 ng AFM_1 /

kg (Anonymous, 2001). In the Turkish Food Codex (Anonymous, 1997), AFM_1 levels in milk were limited to 50 ng/kg, similar to that of EC/Codex Regulations. Due to toxicity, most countries have set up maximum permissible levels of AFM_1 in milk, which varies from 50 ng / kg established by the EU to the 500 ng / kg established by US FDA (European Commission, 2003 and F.D.A., 2011). More restrictive MRLs have been decided by the EU for the presence of AFM_1 in baby food (European Commission, 2004).

Aflatoxin contamination in milk and its products is produced in two ways, either passage of toxins to milk with ingestion of feeds contaminated with aflatoxin, or it results as subsequent contamination of milk and milk products with fungi (Sarimehmetoglu *et al.,* 2003). Aflatoxin M_1 occurrence in milk and dairy products is an important issue because many people consume these products on a daily basis, especially for the growing infant population which depend on milk as a major nutrient.

CONCLUSION

It could be concluded that, the product quality is variable, with some samples showing noticeably more contamination than others. This is probably a reflection of the standards of hygienic measures applied during production and the quality of raw materials used (fermented milk and crushed dried boiled wheat grains), it indicates that with improved hygiene, the microbiological quality of the Kishk could be improved. Kishk samples from Assiut city presented a high incidence of AFM_1 at levels below the limits established by Egyptian regulations. A high percentage of positive samples would be considered inappropriate for human consumption, when considering the tolerance limit adopted by the European Community. The general human exposure to AFM_1 by the consumption of contaminated Kishk is probably non-significant in Egypt. However, the fact that AFM_1 is a potent hepatocarcinogen warrants concern about its occurrence in Kishk, especially those intended for child populations. For this reason, milk and milk products have to be controlled continuously for presence of AFM_1 contamination. It is also extremely important to maintain low levels of AFB in the feeds of dairy animals. In order to achieve this, dairy cow feeds should be kept away from contamination as much as possible and should be checked regularly for aflatoxin and, particularly important, storage conditions of feeds must be strictly controlled.

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أجريت هذه الدراسة لتقييم الجودة الصحية للكشك المنتج في قري محافظة أسيوط. استهدفت الدراسة معرفة مدى احتواء الكشك على بعض البكتيريا والفطريات والسموم الفطرية من عدمه. اشتملت الدراسة علي عدد ستة وثلاثين (٣٦) عينة جمعت بطريقة عشوائية من الكشك المصنع من اللبن الحامضى الجاموسي المنزوع الدسم والمخلوط مع القمح المغلى المجفف المجروش والمباع في محلات العطارة والمصنع بواسطة الفلاحين في قري محافظة أسيوط. تم فحص العينات ظاهريا ثم اجريت عليها الفحوصات البكتيريه والفطرية وكذلك تم تقدير بعض السموم الفطرية بها. أوضحت نتائج الدراسة وجود المكورات المعوية ، البكتيريا الهوائية ، البكتيريا اللاهوائية والخمائر والفطريات فى ٦١.٦ ، ١٠٠ ، ٨٦.١ ، ٧٧.٨ % علي التوالي فى عينات الكشك التى تم فحصها ، وان متوسط العدد الكلى لهذه الكائنات الدقيقة كان ٦.٩٥ x ١٠^{٤} و ٢.٢٦ x ١٠^{٥} و – و ٣.٩ x ١٠^{٤} خلية / جرام في العينات المفحوصة علي التوالي. كما تم تصنيف الفطريات المعزولة الي ***Aspergillus flavus*** و ***Aspergillus niger*** و ***Mucor*** فى ٢٢.٢ ، ١٦.٧ ، ٨.٣ % علي التوالي فى عينات الكشك. باستخدام كروماتوجرافيا الاغشية الرقيقة (Thin layer chromatography) وال ELISA تم قياس مستوي الافلاتوكسين M_1 في عينات الدراسة واظهرت النتائج ان ٢٣.٥٣ % من العينات كانت ايجابية تحتوى السم الفطرى AFM_1 وبتركيز ١٣٥.٣ ± ٦١.٥ نانو جرام / كجم (بطريقة ال TLC) و ١٤٩.٨ ± ٥٦.٥ نانو جرام / كجم (بطريقة ال ELISA). وجود تلوث وبنسب عالية فى عينات الكشك التى تم فحصها انما يدل على عدم اتباع الطرائق الصحية اثناء تصنيع الكشك وحفظه، بالاضافة الى استخدام البان لحيوانات تتغذى على علائق ملوثة بالسموم الفطرية (Aflatoxin) وان السم الفطري AFM_1 يوجد بنسب عالية فى عينات الكشك المصنع والمباع فى اسيوط ولكن بتركيز اقل من المصرح به والمقنن من المنظمات المصرية (Egyptian regulations)، لكن نظرا لخطورة ال AFM_1 كونه يتسبب فى حدوث اورام بالكبد لذلك يجب الحذر عند استخدام الكشك كغذاء ويجب الحصول عليه من مصادر موثوق بها، كما يجب ايضا الحرص على مراقبة اعداده وحفظه بطريقة صحية تمنع تلوثه حتى يصل الى المستهلك بطريقة امنة.