Microbiological monitoring of raw milk and yoghurt samples collected from El-Beida city

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ABSTRACT

A total of 80 raw milk and yoghurt samples were randomly collected from different farms and retail markets of different sanitation levels and examined mycologically and bacteriologically at El-Beida city in Libya. Moulds and yeasts were detected in 80 and 50 % of raw milk and yoghurt samples, respectively, with respective mean values of 4.3 $X10^5 \pm 2.5X\ 10^5$ and 2.1 $X10^4 \pm 1.9\ X\ 10^4$. Aspergillus spp., Cladosporium spp., Mucor spp., Curvularia spp., Penicillium spp., Geotricum spp., Candida spp., Rhodotorula spp., Torulopsis spp. and Saccharomyces spp. could be isolated from both raw milk and yoghurt. Five samples of 40 raw milk once were positive for AFM_1 with the mean value of 5 ppb. While in yoghurt samples, AFM_1 were detected with mean value of 2.2 ppb among 3 samples of 40. Total counts of aerobic bacteria of examined raw milk and yoghurt samples were 6.1 $X\ 10^5 \pm 5.5\ X\ 10^5$ and 6.5 $X\ 10^5 \pm 6.0\ X\ 10^5$, respectively. While the mean coliform counts/ ml were 7.0 $X10^6 \pm 5.0\ X\ 10^6$ and 6.0 $X\ 10^3 \pm 4.0\ X\ 10^3$ for raw milk and yoghurt, respectively. Enterobacteriaceae counts /ml of raw milk and yoghurt samples were 2.6 $X\ 10^6 \pm 5.5\ x10^5$ and 1.5 $X\ 10^4 \pm 1.2\ X\ 10^4$. The economic and public health situation of isolated fungi as well as the control measures for improving the quality of raw milk and milk products were discussed.

***Keywords**: Microbes, raw milk, yoghurt.*

INTRODUCTION

Fresh milk and its products have made a major contibution to infants and adults diet in all countries all over the world .Mould growth on dairy products is quite common . Up to the recent decades, this was considered to be a rather technological than a hygienic problem. Since the discovery of mycotoxins , it has become apparent that some of moulds growing on milk products constitute a health hazared for the consumers if they can produce mycotoxins (Pitt and Hocking, 1997) . Likewise, moulds and their mycotoxins are considered as a potentially major threat to public health and continue to have an extensive impact on the walfare of human and animal populations. Mycotoxins may contaminate dairy products by moulds growing on these products or by the carry-over of mycotoxins occuring in animal feedstuffs ingested by dairy cattle (Egmond, 1983).

Mould contamination not only causes deterioration of food and feeds can adversly affect the health of humans and animals as well since they are capable of producing toxic Metabolites known as mycotoxins causing cases of food poisoning and liver cancer in human (Mossel, 1982; Foster *et al.*, 1983).

Fungi influence the biochemical characters and flavour of the product and its appearance is commercially be undesirable

and often result in down grading of the product.

Coliforms are considered as normal flora of intestinal tract of human and animals. They have been used as indicator organisms for bacteriological quality of milk and its products (I.C.M.S.F. 1986) . Enterobacteriace count is always taken as a definite index of fecal contamination of milk and its products, besides the possible presence of enteric pathogens which may consititute health hazards to the consumers. The most important index of microbiological quality is total bacterial counts, coliforms, yeasts and moulds and detection of specific pathogens and their toxins as recorded by Kwee *et al.* (1986).

The present work introduces necessary information on the hygienic situation of a number of raw milk and yoghurt samples taken from various sources in El- Beida city, Libya. This encompassed the enumeration of total moulds and yeasts, coliforms, Enterobacteriaceae and total aerobes, besides the detection of aflatoxins M_1 and B_1.

MATERIALS AND METHODS

Collection of samples

A total of 80 random samples representing (40) and yoghurt (40) were collected from different shopkeepers in El-beida city in Libya for microbiological evaluation and mycotoxin detection.

Preparation of samples

Raw milk samples were subjected to Storch's test (Lampert 1975) to exclude samples proved to be heat treated . Also collected samples were subjected to Wynter Blyth test for detection of inhibitory substances .Yoghurt samples were thoroughly mixed before beging emulsified in diluent solution .

Microbiological examinations

The prepared samples were subjected to:

1- Aerobic plate count (APC) using standared plate agar (A.P.H.A., 1992).
2- Coliforms content (A.P.H.A. 1985).
3- Enterobacterial count (I.C.M.S.F. 1986).
4- Mould and yeast counts according to Frisvad and Filtenborg, (1989). All mould isolates were identified according to the key of Pitt and Hoching, (1997) while yeast isolates were identified according to Kriger van Rij,(1984) .
5- Detection of aflatoxin M_1 residues in raw milk and yoghurt as well as determination of aflatoxin B_1 in yoghurt. The mycotoxin residues were estimated according to the technique recommended by AOAC (1980).

RESULTS AND DISCUSSION

Results given in Table (1) revealed that moulds and yeasts could be isolated from 80 % of the examined raw milk samples. The lowest counts was $3X\ 10^2$ cfu ml^{-1} , with a mean value of $4.3\ X\ 10^5 \pm 2.5\ X\ 10^5$. Nearly similar findings were reported by Skrinjar *et al.* (1983) and Saubios et *al.* (1991). the obtained results indicated poor hygiene during handling which might lead to technological problems during processing.

Moulds and yeasts were isolated from 50 % of the yoghurt samples Table (1). The minimum Count/ ml. were $1.5\ X\ 10^2 \pm 1.9\ X\ 10^4$. The obtained results agree, to a certain extent, with those reported by Varabioff (1983) & Lalas and Mantes (1984). Lower counts were reported by Jordano – Salina (1984).

The presence of yeasts and moulds in a relatively high counts in examined yoghurt

samples may indicate inefficient pre-heating process during manufacturing, using unsatisfactory sterilized plastic cups in packing or inefficient chilling on storage (Saudi *et al.*, 1989).

Species of *Aspergillus, Cladosporium, Mucor, Curvularia, Penicillium, Pacilomyces and Geotrichium* could be isolated in varying percentages from both examined raw milk and yoghurt samples (Table 2). Many authors obtained the same results (Sharma et *al.* 1993 and Jodral *et al.*, 1991).

Mould and yeast contamination not only causes deterioration of food and feed but also can adversely affect the health of humans. Moreover, fungi influence the biochemical characters and flavour of the product and its appearance is commercially undesirable and often result in down grading of the product.

Results presented in Table (3) indicate that five samples of raw milk (12.5 % of all) were found to be contaminated with aflatoxin M_1 in concentrations of 4 and 6 ppb.This finding comes in agreement with the results reported by El-Sayed *et al.* (2001) and Jalon *et al.* (1994) who found that the level of aflatoxin M_1 in milk was below the permissible limit (10 ug/kg). High levels of aflatoxin M_1 (0.03 and 3.13 ng/ ml) were estimated by Elgerbi *et al.* (2004) and Karaioannoglou *et al.* (1989) who recorded high levels AFM_1 (100 – 130 ug/ Kg) .

On the other hand aflatoxin M_1 residue could be detected in 3 samples of yoghurt at a level ranging from 0.5 - 3 ppb. The results for yoghurt nearly similar with these findings reported by Mohamed (1999) and Polzhafer (1977). Low levels of aflatoxins M_1 were obtained by Galvano *et al.* (1998) in yoghurt samples examined during 1995 in four large Italian cities while high levels were recorded by Hassan (1999).

Aerobic bacterial counts ranged from 5.0 X 10^4 to 2.2 X 10^6 cfu ml^{-1} with a mean value of 6.0X $10^5 \pm 5.5$ X 10^5 and 2.0 X 10^2 to 9.0 X 10^6 cfu ml^{-1} with a mean value 6.5 X $10^5 \pm 6.0$ x 10^5 cfu ml^{-1} for raw milk and yoghurt samples respectively (Table 4) .

Similar findings were reported by Hanaa, (1999), but in contrudication with those of Desmasures, *et al.* (1997). The aerobic bacterial load showed that milk produced was of good microbiological guality ($\leq$20000 / ml.). High aerobic bacterial load in raw milk and yoghurt may be attributed to inadequate hygienic measurs in production or inadequate processing recontamination.

In most foods, the total bacterial count is often, an indiction for the sanitary quality, safety and utility of foods. It may reflects the conditions under which the product is manufactured such as contamination of raw materials and ingredients, the effectivenees of processing and the sanitary conditions of equipment and utensils at the processing plants (I.C.M.S.F., 1986) .

The results in Table (5) showed that the Coliforms were detected in 100 % of raw milk samples and 10 % of yoghurt samples. The coliform counts ranged from 2.0 X 10^2 to 3.0 X 10^7 MPN ml^{-1} with the of mean 7.0 X $10^6 \pm$ 5.0 X 10^6 and from 1.0 X 10^2 to 4.6 X 10^4 MPN ml^{-1} with the mean of 6.0 x $10^3 \pm$ 4.0 X 10^3 MPN ml^{-1} of the examined raw milk and yoghurt samples, respectively .

These results agreed, to a certain extent, with Hanaa (1999); Jayarao and Wang (1999) who reported that coliforms were detected in 62.3 % of milk samples and their counts ranged from 0 to 4.7 $\log_{10}$ cfu/ml with the mean of 3.4 $\log_{10}$ cfu/ml. Lower values were reported by Desmasures *et al.* (1997) .The presence of coliforms in raw foods is generally regarded as direct contamination of foods with fecal material. Coliform bacteria can also gain access directly into milk when cows with subclinical coliform mastitis are milked and have been shown to increase the coliform

counts of milk. Also, several studies have shown that pathogenic *E. coli* comprise a very small percentage of the total *E. coli* present in raw milk (Jayarao and Wang, 1999). Coliforms are considered as normal flora of the intestinal tract of human and animals. They have been used as indicator organisms for bacteriological quality of milk and its products (I.C.M. S.F., 1986). Enteropathogenic *Escherichia coli* has been incriminated as a potential food poisoning agent and is associated with infantile diarrhoea and gastroenteritis in adults.

Result given in Table (6) reveal that enterobacteria could be detected in 100 % of raw milk samples and 25 % of yoghurt samples. The counts ranged from 7.0 X 10^3 to 1.1 x 10^7 cfu ml^{-1} with the mean of 2.6 X 10^6 ± 5.5X 10^5 cfu ml^{-1} in raw milk sample and in case of yoghurt samples counts ranged from 5.0 X 10^2 to 5.0 X 10^4 cfu ml^{-1} with the mean 1.5 X 10^4 ± 1.2 x10^4 cfu ml^{-1}, this result is agreement with Hanaa, (1999).

It is concluded that strict hygienic measures should be applied during production, processing and distribution of milk and its products to avoid contamination. Periodical inspection must be done by specialists on the dairy farms to minimize milk contamination with different types of yeast and moulds. The milk obtained from dairy animals fed on feedstuffs contaminated with aflatoxin B_1 must be rejected. Efficient cleaning and sanitiziation of farm dairy utensiles must be done to improve the quality of raw milk and consequently the related dairy products. Monitoring programs should be more extensive with a particular attention in monitring aflatoxin in milk and milk products. The milk and milk products should be kept under refrigeration and the practice of display at room temperature should be discouraged. Periodical examination of animal feedstuffs for aflatoxin B_1 deems unavoidable and exclude those proved to be highly contaminated.

Table (1) : Statistical analysis of results of mould and yeast counts of examine samples .

Type of examined samples	No. of examined samples	Positive samples		Counts (cfu ml^{-1})			± SE
		No.	%	Min.	Max.	Mean	
Raw milk	40	32	80	3X 10^2	1X10^6	4.3X10^5	±2.5X10^5
Yoghurt	40	20	50	1.5X10^5	3.8X10^5	2.1X10^4	±1.9X10^4

Table (2) : Incidence of isolated moulds and yeasts in examined raw milk and yoghurt samples .

Isolates	Raw milk		Yoghurt	
	No.of isolates	%	No.of isolates	%
Moulds:				
1- Aspergillus				
A .flavus	5	12.5	0	0
* A. Niger*	10	25	2	5
A. Terrus	2	5	0	0
2- *Cladosporium spp.*				
C. herbarum	8	20	0	0
C. sphaerospermum	7	17.5	3	7.5
3- *Mucor sp.*	10	25	6	15
4- *Curvularia spp.*	2	2.5	0	0
5- *Penicillum spp.*				
• *P. corylophilum Dierckx*	6	15	2	5
• *P. implicatum Biourge*	3	7.5	0	0
6- *Paecilomyces sp.*	5	12.5	0	0
7- *Geotricum*	9	22.5	1	2.5
Yeast :				
1- *Candida spp.*				
• *C. parapsilosis*	20	50	8	20
• *C. tropicalis*	6	15	0	0
• *C. Krusei*	2	5	0	0
2- *Rhodotorula spp.*	20	50	9	22.5
3- *Torulopsis spp.*	5	12.5	1	2.5
4- *Saccharomyces spp.*	15	37.5	10	25

Table (3) : Incidence and levels of mycotoxins (ppb) determined in raw milk and yoghurt .

Product	Type of toxin	Positive samples		Range (ppb)	Mean positive (ppb)
		No.	%		
Raw milk	AFM_1	5	12.5	4 - 6	5
Yoghurt	AFM_1	3	7.5	0.5 – 3	2.2
	AFB_1	ND	ND	ND	ND

ND = not detectable

Table (4): Statistical analysis of results of Aerobic bacterial counts (cfu ml^{-1}) of examined samples .

Product	No. of examined sample	Positive samples		Counts (cfu ml^{-1})			
		No.	%	Min.	Max.	Mean	± S.E
Raw milk	40	40	100	$5.0X10^4$	$2.2X10^6$	$6.1X10^5$	$\pm 5.5X10^5$
Yoghurt	40	15	37.5	$2.0X10^2$	$9.0X10^6$	$6.5X10^5$	$\pm 6.0X10^5$

Table (5): Statistical analysis of results of Coliform counts (MPN /ml) of examind samples .

Product	No. of examined sample	Positive samples		Min.	Max.	Mean	± S.E.
		No.	%				
Raw milk	40	40	100	$2.0X10^2$	$3.0x10^7$	$7.0X10^6$	$\pm 5.0X10^6$
Yoghurt	40	4	10	$1.0X10^2$	$4.6X10^4$	$6.0X10^3$	$\pm 4.0X10^3$

Tabl(6) :Statistical analysis of results of Enterobacteria counts(cfu ml of examined samples .

Product	No. of examined samples	Positive samples		Min.	Max.	Mean	± S.E.
		No.	%				
Raw milk	40	40	100	$7.0X10^3$	$1.1X10^7$	$2.6X\ 10^6$	$\pm 5.5X\ 10^5$
Yoghurt	40	10	25	$5.0X10^2$	$5.0X10^4$	$1.5X\ 10^4$	$\pm 1.2X10^4$

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الملخص العربي

الرصد الميكروبي لبعض عينات الحليب الخام والزبادي جمعت من مدينة البيضاء بليبيا

إيمان محمود الدياسطي ، رافع مصطفي الكاسح
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تم في هذه الدراسة فحص 80 عينة من الحليب الخام (40) والزبادي (40) جمعت من مصادر مختلفة بمدينة البيضاء بليبيا. وخضعت للفحص الميكولوجي والبكتيريولوجي. وقد دلت النتائج علي تواجد الفطريات والخمائر بنسبة 80 % ، 50 % في كل من عينات الحليب الخام والزبادي بمتوسط 4.3 × 510 ± 2.5 × 510 ، 2.1 × 410 ± 1.9 ×410 مجموعة لكل مل علي التوالي. وقد كانت الفطريات والخمائر التي تم عزلها تنتمي إلي أجناس الاسبرجيلس، والبنسليوم ، الميكور ، الكلادوسبوريم ، الكلفولاريا ، والبسيلوميسيس ، والجيوتريكم ، والكانديدا ، الروتوريولا ، التيريولوبسيس، السكارواميسيس. وكان عدد العينات الايجابية لوجود الافلاتوكسين م1 5 ، 3 بمتوسط 5 ، 2.2 جزء بالبليون بالنسبة لعينات الحليب الخام والزبادي علي التوالي. وكان متوسط العدد الكلي البكتيري 6.1 × 510 ± 5.5 × 510 و 6.5 ×510 ± 6.0 × 510 مجموعة لكل مل علي التوالي . وأسفرت النتائج عن تواجد ميكروب الكوليفورم في مل الواحد بمتوسط قدرة 7.0 × 610 ± 5.0 × 610 و 6.0 × 310± 4.0 × 310 خلية لكل مل بالنسبة لعينات الحليب الخام والزبادي علي التوالي. كما دلت النتائج علي أن الميكروبات المعوية كانت متواجدة في عينات الحليب الخام والزبادي بمتوسط قدرة 2.6 × 610± 5.5 × 510 و 1.5 ×410 ± 1.2 ×410 خلية لكل مل علي التوالي .