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Chemical and Microbiological Status of Raw Milk Sold at Local Markets

Ahlam El-Leboudy*, Amr A. Amer*, Hussin S. Abo El-Makarem, Ibrahim E.K.

Department of Food Hygiene, Faculty of Veterinary Alexandria University. Egypt

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Correspondence to:*
eleboudy.ahlam@yahoo.com)

ABSTRACT

A total of One hundred samples of raw milk including (50 of cow's milk and 50 of buffalo's milk) were collected randomly from groceries and supermarkets at local markets in Alexandria Governorate for evaluation of chemical, sanitary and microbiological evaluation. Chemical evaluation of examined raw cow's and buffalo's milk samples revealed that the mean values of fat content, protein content, lactose content, solid not fat content and minerals content were (3.57±0.17, 5.5±0.17) (3.8±0.30, 4.03±0.2), (4.5±0.47, 4.9±0.21) (8.91±0.13, 8.9±0.15), (0.50±0.08, 0.74±0.04), respectively. Sanitary evaluation of examined cow's and buffalo's milk samples revealed that the mean values of titratable acidity and pH were (0.16± 0.04, 0.17±0.04) and (6.66 ± 0.07, 6.57± 0.08) respectively. Microbiological evaluation of examined raw cow's and buffalo's milk samples revealed that the mean values of TBC were $2.6x10^5 \pm 0.2x10^5$and $3.2x10^5 \pm 0.2x10^5$ while the mean value total Coliforms count were ($4.12x\ 10^4 \pm 0.3x\ 10^4$) ($4.18x10^4 \pm 0.3x10^4$) respectively. On the other hands the mean values of faecal Coliforms were $3.1x10^4 \pm 0.2x10^4$ and $2.7x10^4 \pm 0.3x10^4$ respectively. Percentage of isolated Coliforms organisms were citrobacter diversus (14.5,18%) citrobacter freundii (16.4,14%), Enterobacter aerogenes (18.2, 20%) Enterobacter cloacae (14.5, 12%) and E. coli (36.4, 36%) in examined cow's and buffalo's samples respectively. Mean value Staphylococcus aureus were $3.0x10^3 \pm 0.3x10^3$ and $3.4x10^2 \pm 0.2x10^3$ in both type examined samples respectively. Mean values of Enterococci count were $0.9x10^3 \pm 0.1x102$ and $1.5x10^2 \pm 0.09x10^3$, respectively. Incidence percent of yeast count were 20% in both type of examined milk while percent of mould count were 24 and 20% in examined milk samples, respectively. Public health significance of different microbial contamination as well as the recommended hygienic measures for production of high quality raw milk was discussed.

1. INTRODUCTION

Milk is considered as nature's single most complete food and is definitely as one of the most valuable and regularly consumed foods. But at the same time, it is highly vulnerable to bacterial contamination and hence is easily perishable (OECD, 2005).

Chemically, milk is a complex mixture of fat, protein, carbohydrates, minerals, vitamins and other miscellaneous constituents dispersed in water, make it a complete diet (Haug et al. 2007).

Titratable acidity is a measure of freshness and bacterial activity in milk (Popescu and Angel 2009) reported that high quality milk essentially needs to have less than 0.14 percent acidity. The acidity of the raw milk samples varied largely from one samples to another during the storage period.

The total bacterial count alternatively known as total viable count that measures the amount of microbial load as well as bacterial contamination in milk (Swanson et al. 1992).

Coliforms count provides an indication of both the effectiveness of cow preparation procedures during milking and the cleanliness of the cow's environment. The major source of Coliform bacteria in bulk tank milk is the transportation of soil from teats and udders into

the milking machine. Coliforms can also incubate on residual films of milking equipment; Coliform counts less than 10 cfu/ml indicate excellence in both pre-milking hygiene and equipment sanitation (Reinemann, 2002).

The presence of Staphylococcus aureus in raw milk may be due to cow's mastitis, handlers and from hygiene deficient. Its presence in foods represents a risk to human health, causing a public health problem, as foodborne intoxication (Quintana and Carneiro 2006).

High level of Staphylococcus aureus in milk means that their microbial contamination index can be used to judge the quality, as well as the sanitary conditions of its production and the health of the herd (Guerreiro et al., 2005).

Mould are important in milk, which is used for the manufacture of dairy products. The presence of wild types of moulds is undesirable as they may influence the organoleptic characteristics of the dairy products, they can produce mycotoxins and represent a potential health risk (Wouters et al., 2002)

The presence of yeasts themselves are not commonly the cause of defect in milk and dairy products unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeast or fruity flavour and obvious gas (Davis and Wilbey, 1990)

Therefore, the objectives of this study were design to determine chemical, sanitary and microbiological status of cow's and buffalo's milk samples that randomly collected from groceries and supermarkets in Alexandria Governorate, Egypt. Moreover, study the prevalence of foodborne pathogens, especially Entero pathogenic. E, coli, S. aureus, enterococci and yeast and mould in examined raw milk samples.

2. MATERIAL AND METHODS

1. Collection of samples:

One hundred samples of raw milk including (50 of each cow's and buffalo's milk) were collected randomly from groceries and supermarkets at local markets in Alexandria governorate.

2. The Examined samples were subjected to the following examinations:

2.1. Chemical evaluation examined raw milk samples using milk scan (lacto – scan)

2.2. Sanitary evaluation of examined raw milk samples:

2.2.1. Determination of titratable acidity percent according to (AOAC, 1990).

2.2.2. Determination of pH value using pH meter according to (APHA, 1992).

2.3. Microbiological evaluation of examined raw milk samples:

2.3.1. Preparation of serial dilution (APHA, 1985).

One ml of well mixed milk sample was added separately to 9 ml of sterile saline solution then thoroughly mixing for preparation of 10-fold serial dilutions, from which the decimal dilutions were prepared to detect:

2.3.2. Total bacterial count (APHA, 1992).

2.3.3. Coliforms count (ICMSF, 1978).

2.3.4. Faecal coliform (ICMSF, 1978).

2.3.5. Counting of pathogenic E.coli (Coia, at el 2001)

2.3.6. **Enumeration and isolation of Staphylococcus aureus (Bailey and Scott, 1998).**

2.3.7. Enterococcus count. (Franz, at el, 1999)

2.3.8. Total Yeasts and total moulds count (Bailey and Scott, 1998).

3. RESULTS AND DISCUSSION

Table (1): Mean of chemical composition in examined raw cow's and buffalo's milk samples.

Parameters	Cow milk	Buffalo's milk
	Mean ±SEM	Mean ±SEM
Fat	3.57±0.17	5.5±0.17
Protein	3.8±0.30	4.03±0.2
Lactose	4.5±0.47	4.9±0.21
SNF	8.91±0.13	8.9±0.15
Minerals	0.50±0.08	0.74±0.04

Table (2): Comparison between the obtained results of chemical composition of the examined raw cow's and buffalo's milk samples with fat percent and SNF of the Egyptian Standards **(ES, 154/1/2005).**

Sources	No. of examined samples	Fat%				SNF%			
		Compatible samples		Incompatible samples		Compatible samples		Incompatible samples	
		No.	%	No.	%	No.	%	No.	%
Cow milk	50	45	90	5	10	50	100	0	0
Egyptian Standards (154|1|2005)		**Not less than 3**				**Not less than 8.25**			
Buffaloe milk	50	40	80	10	20	41	82	9	18
Egyptian Standards (154|1|2005)		**Not less than 5.5**				**Not less than 8.75**			

Table (3): Mean values of titratable acidity and pH value of examined samples of raw (cow's and buffalo's) milk.

Parameters	Range	Mean value of Cow milk	Range	Mean values of Buffaloe milk
Acidity percent	0.13 - 0.18	0.16± 0.04	0.15- 0.19	0.17 ± 0.04
pH values	6.20 - 6.99	6.66± 0.07	6.26 - 6.97	6.57 ± 0.08

Table (4): Microbiological evaluation of examined raw cow's and buffalo's milk samples.

	Cow milk			Buffaloe milk		
	No.	%	Mean ± SEM	No.	%	Mean ± SEM
TBC	**50**	**100**	$2.6x10^5 \pm 0.2x10^5$	**50**	**100**	$3.2x10^5 \pm 0.2x10^5$
Coliforms	**50**	**100**	$4.12x10^4 \pm 0.3x10^4$	**49**	**98**	$4.18x10^4 \pm 0.3x10^4$
Entero Pathogenic E.coli	**49**	**98**	$3.49x10^3 \pm 0.2x10^2$	**50**	**100**	$3.46x10^3 \pm 0.3x10^3$
F. Coliforms	**25**	**50**	$3.1x10^4 \pm 0.2x10^4$	**25**	**50**	$2.7x10^4 \pm 0.3x10^4$
S. aureus	**50**	**100**	$3.0x10^3 \pm 0.3x10^3$	**50**	**100**	$3.4x10^3 \pm 0.2x10^3$
Enterococci	**50**	**100**	$0.9x10^3 \pm 0.1x10^3$	**50**	**100**	$1.5x10^3 \pm 0.09x10^3$
Yeast	**10**	**20**	$0.7x10^3 \pm 0.06x10^3$	**10**	**20**	$0.8x10^3 \pm 0.1x10^3$
Mould	**12**	**24**	$0.29x10^3 \pm 0.03x10^3$	**10**	**20**	$0.30x10^3 \pm 0.03x10^3$

TBC= total bacterial count F= faecal coliforms S= Staphylococcus aureus

Table (5): Frequency distribution of identified yeasts isolated from examined raw cow and buffaloe milk samples.

Isolated yeast strains	Cow milk		Buffaloe milk	
	NO	%	No	%
Candida albicans	5	31.25	6	33.3
Candida krusei	3	18.75	3	16.7
Candida pseudotropicalis	3	18.75	4	22.2
Candida tropicalis	2	12.5	2	11.1
Rhodotorula glutinis	2	12.5	2	11.1
Rhodotorula rubra	1	6.25	1	5.6
Total	16	100	18	100

Table (6): Frequency distribution of identified mould isolated from examined raw cow and buffaloe milk samples.

Isolated mould strains	Cow milk		Buffaloe milk	
	No	%	No	%
Aspergillus flavus	4	20	2	12.5
Alternaria alternata	2	10	1	6.25
Aspergillus fumigatus	2	10	2	12.5
Aspergillus niger	2	10	2	12.5
Cladosporium clavocipus	4	20	3	18.75
Geotrichum species	2	10	1	6.25
Penicillium species	3	15	3	18.75
Rhizopous spp.	1	5	2	12.5
Total	**20**	**100**	**16**	**100**

3.1. Chemical evaluation of examined raw cow's and buffalo's milk samples.

Milk composition can be very variable, and it depends on numerous of factors such as breed, health of lactating dairy animals, lactation period, manner and type of nutrition, season of the year, manner of milking (manual or automatic), as well as age and number of lactation, and finally on the individual itself (body mass, moving, etc.) **(**Tratnik, 1998).

Table (1) showed that the mean values of chemical parameters of examined raw cow's and buffalo's milk samples were 3.57±0.17 and 5.5±0.17 for fat, 3.8±0.30 and 4.03±0.2 for protein, 4.5±0.47 and 4.9±0.21 for lactose, 8.91±0.13 and 8.9±0.15 for SNF and 0.50±0.08 and 0.74±0.04, for mineral content, respectively.

Egyptian Standards (2005) stated that raw cow's milk fat percent and SNF should be not less than 3 and 8.25 %, respectively. According to this standard, 90 % of examined raw cow milk samples were compatible with fat standard and all examined raw cow milk was compatible with SNF standard.

Egyptian Standards (2005) stated that fat percent and SNF of raw buffalo's milk should not less than 5.5 and 8.75 %, respectively. According to this standard80 % of examined raw buffalo's milk samples were compatible with fat standard and 82 % of examined raw buffalo's milk were compatible with SNF standard.

District, climatic conditions and lactation periods are known as seasonal changes which have influences on the milk composition. Milk fat is the most variable component among the milk contents that affected by these changes. Especially, there is a negative correlation between environmental temperature the amount of milk fat and protein content. When temperature increases the solid fat tends to decrease. The light-to-dark ratio can also induce marked changes in milk yield and composition. In

fact, a high light-to-dark ratio leads to a reduction in fat and protein contents of milk, probably as a consequence of a greater secretion of prolactin whose concentration in plasma is higher in the summer than in the winter (Casati et al.1998).

The more pronounced variation in milk fat was due to outdoor grazing in summer, bar feeding in winter and adulteration by partial skimming by farmers. There were many important differences between the feeds composition which were given to animals during these two periods (Yetismeyen, 2000). Probably due to the fact that the feed diet was based on hay during the winter months the herbage was not available. Increased feeding frequency of low fiber, high grain diets increase milk fat levels (Waldner et al. 2005).

3.2. Sanitary evaluation of examined raw cow and buffaloe milk sample:

Table (2) revealed that the mean values of titratable acidity in examined raw cow and buffaloe milk samples were 0.16± 0.04 and 0.17 ± 0.04, respectively. While the mean values of pH values in examined raw cow and buffaloe milk samples were 6.66± 0.07 and 6.57 ± 0.08, respectively.

Titratable acidity of milk has long been recognized and employed as an indicator of quality (Griffiths et al. 1988). It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity (O'Mahony, 1988).

The difference in acidity was due to metabolic activities of microbes. Longer period of storage is also responsible for higher titratable acidity which in turn effects on the bacteriological quality of raw milk (Mennane et al. 2007).

PH value the milk samples is related to acidity. Lower pH is indicative to higher developed acidities due to action of microbes whereas higher pH values were obtained when cattle were suffering from Mastitis (**Walstra et al. 2006).**

3.3. Microbiological evaluation of examined raw cow and buffaloe milk samples:

Raw milk is a well-known as good growth medium that supports the growth of several microorganisms because of its high water content, nearly neutral pH and variety of available essential nutrients that renders it as one of the best media for microbial growth and multiplication (Soomro et al. 2002).

The bacterial contamination of milk not only reduces the nutritional quality but also the consumption of such milk leads to threatens health of the society. Microorganisms may contaminate milk at various surrounding stages of procurement, processing and distribution. The ill health of cow and its environment, improperly cleaned and sanitized milk handling equipment, and unhygienic workers who milk the cow could serve as a sources of microbial contamination (Fadaei, 2014).

Data presented in Table (4) showed that the incidence percent of total bacterial, Coliforms, faecal Coliform, Staphylococcus aureus, Enterococci, yeast and mould count in examined raw cow milk samples were 100, 100, 50, 100, 100, 20 and 24 % respectively, with a mean value of $2.6x10^5 \pm 0.2x10^5$,$4.12x10^4 \pm 0.3x10^4$,$3.1x10^4 \pm 0.2x10^4$, $3.0x10^3 \pm 0.3x10^3$,$0.9x10^3 \pm 0.1x10^3$, $0.7x10^3 \pm 0.06x10^3$ and $0.29x10^3 \pm 0.03x10^3$, respectively. On the other hand the incidence percent of total bacterial, Coliforms, faecal Coliform pathogenic E.coli, Staphylococcus aureus, Enterococci, yeast and mould count in examined raw buffaloe milk samples were 100, 98, 50, 100, 100, 20 and 20 %, respectively, with mean a value of $3.2x10^5 \pm 0.2x10^5$, $4.18x10^4 \pm 0.3x10^4$, $3.49x10^3 \pm 0.2x10^3$, $3.46x10^3 \pm 0.3x10^3$,$2.7x10^4 \pm 0.3x10^4$, $3.4x10^3 \pm 0.2x10^3$, $1.5x10^3 \pm 0.09x10^3$, $0.8x10^3 \pm 0.1x10^3$and $0.30x10^3 \pm 0.03x10^3$, respectively.

Bacterial contamination of raw milk can originate from different sources as: air, milking equipment, feed, soil, faeces and grass. The

number and types of microorganisms in milk immediately after milking are affected by several factors as animal and equipment cleanliness, season, feed and animal health. It is hypothesized that differences in feeding and housing strategies of cows may influence the microbial quality of milk (Coorevits et al. 2008).

Milk with high bacterial contamination has a poor keeping quality that may cause intestinal disturbances especially in infants as it considered the main diet for them (karmen G. T0 and slavica 2008)

The presence of coliform organisms in milk indicates unsanitary conditions during production, processing and storage. Hence their presence in large number in dairy products gave an indication that about the presence of potentially hazard in consumers' health (Godefay and Molla,2000).

Pathogenic E.coli most recently has become serious threat to dairy incidence ranging from diarrhea to potentially faecal hemolytic uramic syndromes (Coia et al. 2001).

Milk can be contaminated by Staphylococcus aureus through infection of mammary glands or through from bad hygienic habits, as coughing or sneezing and neglecting of cleanliness. (Murray et al. 2006)

The presence of Enterococci in large numbers may constitute a public health hazard and may actas a cause of food poisoning because of their ability to produce extracellular toxic metabolites (Roushdy et al. 1997).

Presences of yeasts themselves are not commonly the cause of defect in milk and dairy products unless they ferment lactose. In this case, they can grow rapidly and produce a characteristic yeasty or fruity flavour and obvious gas (Davis and Wilbey, 1990). They also produce metabolites, e.g. short-chain fatty acids and other compounds, with a known toxic effect against undesirable microorganisms in the intestinal tract (Jakobsen and Narvhus, 1996).

Moulds are represent an important role in milk, as it used for the manufacture of some dairy products. On the other hand, the presence of wild types of moulds is undesirable as they may influence the organoleptic characteristics of the dairy products; they can produce mycotoxins and represent a potential health risk (Wouters al. 2002). Aflatoxins are on of most serious mycotoxins it produced as carcinogenic, teratogenic and mutagenic secondary metabolites by some species of genus Aspergillus (Frisvad et al. 2005).

The percentage of the most prevalent yeast species that could isolated from examined raw cow milk samples were identified as candida albicans 5 (31.25%), Candida krusei 3 (18.75%), Candida pseudotropicalis 3 (18.75%), Candida tropicalis 2 (12.5%), Rhodotorula glutinis 2 (12.5%) and Rhodotorula rubra 1(6.25%) respectively. While, for raw buffaloe milk samples were identified ascandida albicans 6 (33.3%), Candida krusei 3 (16.7%), Candida pseudotropicalis 4 (22.2%), Candida tropicalis 2 (11.1%), Rhodotorula glutinis 2 (11.1%) and Rhodotorula rubra 1(5.6%), respectively, table (5)

The most prevalent moulds species that could be isolated from examined raw cow milk were identified as Aspergillus flavus 4 (20%), Aspergillus Fumigatus 2(10%), Aspergillus Niger 2 (10%), Alternaria alternata 2 (10 %), Cladosporium Clavocipus 4 (20%), Geotrichum species 2 (10 %), Penicillium spceis 3 1(5%), and Rhizopous species 1(5%), respectively. The predominant mould species in raw buffalo'smilk were identified asAspergillus flavus 2 (12.5%), Aspergillus Fumigatus 2(12.5 %), Aspergillus Niger 2 (12.5%), Alternaria alternata 1 (6.25 %), Cladosporium Clavocipus 3 (18.75%), Geotrichum species 1 (6.25 %), Penicillium spceis 3 (18.75%), and Rhizopous species 2(12.5%), respectively, Table (6).

High prevalence of different udder pathogen among dairy animals may attributed to the lack of sanitary conditions that adapted in dairy farm So, restriction to application of hygienic measures in dairy farms as well as quality control and quality assurance programs should be adopted to achieve safety and quality of produced raw milk.

In conclusion the overall hygienic conditions surrounding the production and handling of milk should be monitored.

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