Microbiological Studies on some Spoilage and Food-Poisoning Microorganism in Raw Milk.

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Abstract:

A total of 35 of raw milk samples were collected from street vendors and different supermarkets in Ismailia and Port Said cities and subjected to sensory, chemical and microbiological evaluation**.** The results showed that acidity % was 0.38±0.02 and Fat% was 2.76±0.07. Microbiologically the mean value of total *Staph. aureus* was 6.37±0.15 (Log_{10}) cfu/ml,. Salmonellae failed to be detected. While the mean value of total Enterobacteriacaea count was 7.91±0.27 (Log_{10}) cfu/ml. Also, the mean value of total Coliform count was 7.65± 0.35(Log_{10}) c fu/ml, and the mean value of total yeasts and mold count was 3.26 ± 0.09 (Log_{10}) cfu/ml. Finally, significance of food spoilage and poisoning isolates, possible sources of contamination and some recommendations to improve the quality of this product were discussed.

Introduction:

Milk is a complex natural liquid, and a decent medium for growth of different species of microorganisms. It is difficult to maintain a strategic distance from contamination of milk with microorganisms because of its unhygienic creation ***(Rogelj, 2003).*** There are different sources for bacterial contamination of raw milk such as air, soil, animal feed, feces and dairy equipment. Differences in feeding and housing strategies of cows may influence the microbial quality of milk ***(Coorevits et al., 2008).*** Milk and milk products borne outbreaks represent 2-6% of bacterial food-borne outbreaks reported by surveillance systems in a several nations. *Staphylococcus aureus* is the most frequent pathogen connected with outbreaks (85.5%) followed by Salmonella (10.1%) ***(De Buyser et al., 2001).*** *Staphylococcus aureus* is one of the most common causes of bacterial food poisoning outbreaks. Likewise it is a major causative agent of clinical and subclinical mastitis of dairy ruminants. Staphylococcal food-poisoning commonly occurred in milk and milk products ***(Le Loir et al., 2003)***. Food and Drug Administration reported that soft cheeses produced using raw milk can bring about serious infectious diseases like salmonellosis ***(Reed et***

al., 2011). Enterobacteriaceae total count is very important index of fecal contamination of milk and milk products, presence of enteric pathogens may constitute general well-being dangerous to the customers ***(Kwee et al., 1986).*** Coliforms are routinely utilized as marker to the nature of milk and milk products as a few individuals from coliforms are responsible for the development of objectionable taints in milk and its products rendering them of substandard quality or even unmarketable ***(Yabaya and Idris 2012).*** Yeasts and mold in milk and dairy products are undesirable even if found in few numbers as they bringing about objectionable changes that render the products of low quality ***(Abdel hameed, 2011)***. Therefore, the present study was conducted to evaluate sensory, chemically and bacteriologically milk traded in Ismailia and Port Said cities.

Material and Methods

1. Samples Collection: A total of 35 raw milk samples were collected from street vendors and different supermarkets in Port Said and Ismailia cities, Egypt. The collected samples were directly transported in an ice box to the laboratory to be examined without delay. All samples were subjected to (sensory, chemical and microbiological examinations).

2. Preparation of samples *(ISO, 2003):* 25 ml of each sample were added to 225 ml 0.1% sterile buffered peptone water (oxoid) into sterile stomacher bags of approximately 500 ml capacity. Samples were blended in a seward stomacher (400 R/UK) for 2 min. to prepare a 1:10 dilution. One ml of the original dilution was transferred serially into sterile test tubes containing 9 ml of 0.1% sterile buffered peptone water to obtain a final dilution of 10^7.

3. Sensory examination: Samples were evaluated according to **(American Dairy Science Association, *ADSA, 1987)*,** by using a system of 20 points (10 points to flavor, 5 points to body and texture and 5 points to appearance)**.**

4. Chemical examination:

4.1. Determination of titratable acidity *(AOAC, 1995).*

4.2. Determination of fat content using Gerber's method *(FAO, 1997).*

5. Microbiological Examination:

5.1. Enumeration of *Staphylococcus aureus* organisms: The technique used in this study was carried out according to the method described by ***(Deibel and Herrtman, 1984)*** and ***(Baired Parker, 1962)***

5.2.Biochemical identification of *Staphylococcus aureus* by:

1- Catalase activity test *(Taylor and Achanzer, 1972)*

2- Coagulase test *(Cruickshank et al., 1975)*

3- Mannitol test *(Cowan and Steel, 1974)*

5.3. Isolation of Salmonellae: The technique used in this study was carried out according to the method described by ***ISO (2002b)***

5.4. Isolation and Identification of Enterobacteriaceae: The technique used in this study was carried out according to the method described by ***(ISO, 2004)*** and ***(Konemann et al., 1993).***

5.5. Total coliform count: The technique used in this study was carried out according to the method described by ***(FDA, 2002).***

5.6. Total yeasts and mold counts: The technique used in this study was carried out according to the method described by ***(APHA, 1992)***.

Results

Table (1): *The sensory scores of milk samples:*

Flavour criticisms	**Score**			**No. of samples**			**%**		
No criticism	10			20			57.14		
	Intensity of defect								
	Slight			Definite			pronounced		
	score	No	%	Score	No	%	score	No.	%
Bitter	9	10	28.6	7	4	11.42	5	0	0
Rancid	4	1	2.9	2	0	0	0	0	0
Body & texture	Score			No. of samples			%		
No criticism	5			30			85.7		
	Intensity of defect								
	Slight			Definite			pronounced		
	score	No	%	Score	No.	%	score	No.	%
Grainy/gritty	4	4	11.42	3	1	2.9	2	0	0
Appearance	Score			No. of samples			%		
No criticism	5			21			60		
	Intensity of defect								
	Slight			Definite			pronounced		
	score	No	%	Score	No.	%	score	No.	%
Atypical colour	4	13	37.14	3	1	2.9	2	0	0

Table (2): *Statistical analytical results of acidity% in examined milk samples (n = 35).*

Examined Sample	**Minimum**	**Maximum**	**Mean ± S.E.**
Milk	0.20	0.70	0.38 ± 0.02

n means the number of examined samples.

Table (3): *Statistical analytical results of Fat% in examined milk samples:*

Examined samples	Minimum	Maximum	Mean ± S.E.	E.S (2005)	Samples not conform E.S.2005	
					No.	%
Milk	2.00	3.50	2.76 ± 0.07	Not less than 3%	16	45.7

Table (4): *Statistical analytical results of total Staphylococcus aureus count (Log_{10}) cfu/ g in the examined samples*

Examined Samples	Count /ml or gm			E.S.	Samples not conform E.S (2005)	
	Minimum	Maximum	Mean ± S.E.	(2005)	No.	%
Milk	5.3	8.06	6.37 ± 0.15	Not more than 10 cfu/ml.	35	100.0

Table (5): *Positive and negative samples for Salmonella in examined samples.*

Samples	+ve samples		-ve samples		E.S.	Samples not conform E.S.(2005)	
	No.	%	No.	%	(2005)	No.	%
Milk	0	0	35	100	Free from salmonella	0	0

Table (6): *Statistical analytical results of total Enterobacteriaceae count (Log_{10}) cfu/ g in the examined samples (n = 35).*

Examined Samples	Count /ml or gm			E.S. (2005)	Samples not conform E.S (2005)	
	Minimum	Maximum	Mean ± S.E.		No.	%
Milk	5.45	9.9	7.91 ±0.27	Not more than 10 cfu/ml.	35	100.0

Table (7): *Incidence of isolated Enterobacteriacaea from the examined samples:*

Strains	milk	
	No.	%
Citrobacter fruendii	4	11.43
E.coli	5	14.29
Enterobacter aerogenes	7	20.00
Kelbsiella oxytoca	3	8.57
Kelbsiella pneumoniae	4	11.43
Proteus vulgaris	6	17.14
Proteus mirabilis	6	17.14

Table (8): *Statistical analytical results of total coliform count (Log_{10}) cfu/ g in the examined samples (n = 35).*

Examined Samples	positive samples		Count /ml or gm			E.S (2005)	Samples not conform E.S (2005)	
	No.	%	Minimum	Maximum	Mean ± S.E.		No.	%
Milk	34	97.14	0	9.75	7.65 ±0.35	Not more than 10 cfu/ml.	34	97.14

Table (9): *Statistical analytical results of total yeasts &mold count (Log_{10}) cfu/ g in the examined samples (n = 35).*

Examined Samples	Count /ml or gm			E.S. (2005)	Samples not conform E.S (2005)	
	Minimum	Maximum	Mean ± S.E.		No.	%
Milk	2.18	4.18	3.26 ± 0.09	Not more than 10 cells/ml yeast & 400 cells/ml moulds.	35	100.0

Discussion

Sensory examination: Sensory evaluation of milk and dairy products is used to measure their quality, scoring and helping in pointing out the defects that may be found to improve their acceptability and marketing . Quality scorecard for sensory evaluation should be made for each examined dairy product which determined by comparing the characteristics of

each product with their accepted standard of perfection ***(Bodyfelt et al., 1981).*** Data presented in Table (1) showed that 57.14% of milk samples with no flavor criticism. While the incidence of slight defect was 28.6% and 2.9% for bitter and rancid, respectively. And 85.7 % of milk samples with no body and texture criticism. While the incidence of slight defect was 11.42% for grainy. While 60 % of milk samples with no appearance criticism. While the incidence of slight defect was 37.14 % for atypical colour.

Chemical:

1. Titratable acidity

Data presented in table (2) showed that the mean value of the titratable acidity in examined milk samples was 0.38±0.02. Lower findings were reported by ***(Asif and Sumaira, 2010 and Teshome et al. ,2015)***.

2. Fat %:

Data presented in table (3) showed that the mean value of Fat% in examined milk samples was 2.76±0.07. Nearly similar results were recorded by ***Stanescu et al. (1992); Mutukumira et al. (1996)*** and ***Bille et al. (2009).*** On contrary lower findings were reported by ***Enab et al. (2009)***. On the other hand higher findings were reported by ***Simundic (1991); Aoyama et al. (1992); Resende-de-Souza et al. (1997); Kuczaj (2001) and Han et al. (2007).*** According to ***Egyptian Standard (2005)*** fat % of the cow`s milk must be not less than 3%, so 45.7% of examined milk samples failed to success ***E.S. (2005).***

Microbiologically:

1. *Staphylococcus aureus*

Staphylococcus aureus in dairy food is an index of its contamination from workers sharing in production and handling. Besides, enterotoxigenic *Staphylococcus aureus* strains may find chance to develop and duplicate in the food leading to food poisoning among purchasers ***(Abdel hameed and El malt, 2009).***

Data illustrated in table (4) showed that the mean value of total Staph. aureus was 6.37±0.15 (log) cfu/ml, in milk. Lower findings were reported by ***Kiymet et al., (2010); Lílian et al. (2011); Mohammed et al., (2012)*** *and* ***Nashwa et al. (2014)***. While higher findings were recorded by ***Oluwafemi and Lawal (2015)***. According to ***Egyptian Standard (2005)*** 100% of examined milk samples failed to success ***E.S (2005)*** (total Staphylococcal count must be < 10cfu/ml).

2. Salmonellae

Salmonella is one of the most essential enteric food-borne pathogen whose detection in the food constitutes a serious health hazard. Many outbreaks of human disease caused by utilization of contaminated raw or deficiently heat treated milk or their dairy products ***(Ellis et al., 1998)***. Data in table (5) proved that *salmonella spp.* Failed to be detected in all the examined samples. Nearly similar results were recorded by ***Mohamed***

and Gihan (2014) and ***Elbagory et al. (2015)***. Higher findings were given by ***Baloch et al. (2015)*** and ***Mossie et al. (2016)***.

According to ***Egyptian Standards (2005)*** all examined samples of milk success to conform ***E.S. (2005)*** which stated that the milk and dairy products must be free from salmonella).

3. Enterobacteriaceae:

Enterobacteriaceae spp. have been shared in many cases of food poisoning outbreaks, other than being involved in food borne gastroenteritis. It is viewed as a decent pointer of possible fecal pollution of dairy products ***(Benkerroum et al., 2004).***

The data presented in table (6) showed that total Enterobacteriaceae count in examined milk samples was 7.91±0.27 (log) cfu/ml. The results are totally different from those reported ***Abebe et al. (2012) and Cervinkova1 et al. (2013)***. According to ***Egyptian Standards (2005)*** total Enterobacteriaceae count must be not more than 10 cfu/ml in milk, therefore 100% of examined milk were not confirmed ***E.S. (2005)***.

Data presented in table (7) showed that incidence of Enterobacteriacea isolated from the examined samples were *Citrobacter fruendii* (11.4%) *E.coli (*14.3%) *Enterobacter aerogenes* (20%) *Kelbsiella oxytoca* (8.6%) *Kelbsiella pneumonia* (11.4%) *Proteus vulgaris* (17%) *Proteus mirabilis* (17%). These results were not agreed with that recorded by ***Yagoub et al. (2005); Donkor et al. (2007) and Elbagory et al. (2016)***.

4. Coliform

Detection of coliforms in raw milk was the most part viewed as immediate tainting of food with fecal material ***(Kirk. 2005).***

The data reported in table (8) showed that the mean value of total coliform count was 7.65± 0.35 (Log_{10}) cfu/ml, of examined milk samples. These results were disagree with those reported by ***Abebe et al. (2012) and Oluwfemi and Lawal (2015)*** as they show lower results of coliform level. According to ***Egyptian Standards (2005)*** total coliform count must not be exceed 10 cfu/ml in milk. Therefore 97.14%, of examined milk samples were not confirmed ***E.S. (2005)***.

5. Yeasts & mold

Contamination of dairy products with yeasts and mold is undesirable even found in few numbers, since they quickly developed in an extensive variety of temperature, pH and humidity. Their growth resulting in objectionable changes that render the product either of inferior quality or even unmarketable ***(Robinson and Tamime, 2002).***

Data illustrated in table (9) revealed that the mean value of total yeast & mould count in the examined milk samples was 3.26± 0.09, 5.01±0.07, 4.5 ± 0.08, 2.48 ± 0.29 (Log_{10}) cfu/ml. Lower findings were

reported by ***Karmen and Slavica (2008)***. On the other hand higher findings were given by ***Teshome et al. (2014)*** and ***Oluwafemi and Lawal (2015).*** According to ***Egyptian Standards (2005)***, all examined samples were not confirmed were not confirmed were not confirmed ***E.S. (2005)*** which reported that Milk should not contain more than 400 cells/ml for yeasts and 10 cells/ml for mold**.**

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"دراسات ميكروبيولوجية عن بعض الميكروبات المسببة لكل من الفساد والتسمم الغذائي في اللبن الخام "

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اجريت الدراسة علي (35 عينة من اللبن الخام) جمعت من اماكن مختلفة مثل الاسواق والمحلات والباعة الجائلين في مدينتي الاسماعيلية وبورسعيد, وتم تقييم هذه العينات طبقا لمعايير الفحص الظاهري والفحص الكيميائي وايضا طبقا لمعايير الفحص الميكروبيولوجي ثم تم مقارنة هذه النتائج بالمواصفات القياسية المصرية لتقييم مدي جودة العينات.

وقد أظهرت النتائج ما يلي:- معظم العينات كانت سليمه ظاهريا وانه برغم تواجد الميكروبات الا انه لم يؤثر ذ لك سلبا علي الصفات الظاهرية للعينات موضع الفحص وكان متوسط نسبة الدهون 2.76±0.07 و متوسط نسبة الحامضية كانت 0.38±0.02. . وتواجد ميكروب المكور العنقودي الذهبي بنسبة100% و بمتوسط, 6.37±0.15 أما عن ميكروب السالمونيلا فلم يتواجد في أي من العينات التي تم فحصها. كما أوضحت النتائج ان تواجد الميكروبات المعويه كانت بنسبة100% وبمتوسط 7.91±0.27. كما أظهرت النتائج ان نسبة العينات الايجابية لميكروبات المجموعة القولونية كانت 97.14% و بمتوسط 7.65±0.35. وكان العدد الكلي للخمائر والفطريات بنسبة100%. و بمتوسط 3.26±0.09, 5.01 .هذا وقد تم مناقشة الأهمية الصحية للميكروبات المعزولة و المسببة للفساد والتسمم الغذائي، وطرق تلوثها و إعطاء بعض التوصيات لتحسين جودتها وتشديد الرقابة عليها.