

Dept. of Food Hygiene, Animal Health Research Institute,
Assiut, Regional Laboratory.

**CHEMICAL AND MICROBIOLOGICAL ANALYSIS
TO EVALUATE THE SANITARY CONDITION
OF RAW MILK IN ASSIUT**
(With 3 Tables)

By

EMAN KORASHY and NAHED MOHAMED

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التحليل الكيميائي والميكروبيولوجي لتقييم الحالة الصحية للبن الخام في أسيوط

إيمان قرشى ، ناهد محمد وهبه

يشغل الكثيرون من أصحاب مزارع ومصانع الألبان بجودتها وخلوها من الملوثات البكتيرية والفطرية الضارة لما لهذه الألبان من أهمية خاصة كغذاء كامل يتناسب مع المراحل العمرية للصغار والكبار. لذا تم جمع خمس وسبعين عينة من اللبن الخام من بعض مزارع الألبان بمحافظة أسيوط لتقييمها باختبارات مراقبة الجودة التي تعتمد على زيادة الحموضة والانزيمات وكذلك العدد الكلي للميكروبات الهوائية والقولونية والخمائر والفطريات وكذلك البحث عن تواجد البكتريا اللاهوائية المتحوصلة. بالنسبة لنتائج التحليل الكيميائي فقد كان متوسط الأيون الهيدروجيني ٦,٨ ومتوسط النسبة المئوية للحموضة هو ٠,١٦% وعند تقييم حموضة العينات باختبارات التخثر أثناء الغليان والترسيب باستخدام الكحول الايثيلي بتركيز ٦٨% كانت ٣٦% و ٤١,٣% من العينات إيجابية على التوالي. وعند استخدام اختبار اختزال صبغة الريزازيورين ثبت أن ١٣,٣% من العينات حالتها جيدة، ٢٤% بحالة متوسطة، ٢٨% حالتها سيئة و ٣٤,٧% سيئة جداً وفي نفس الوقت كانت ١٦، ٣٢ و ٥٢% من العينات حالتها جيدة، متوسطة وسيئة عند استخدام اختبار الكاتاليز البسيط على التوالي. وأثبتت اختبارات مراقبة الجودة أن متوسط العدد الكلي للبكتريا الهوائية والقولونية والخمائر والفطريات هو 1.0×4 ، 1.0×9 ، 1.0×12 ، 1.0×3 على التوالي أما البكتريا المتحوصلة اللاهوائية فقد سجلت ٢٥,٣% وقد تم في هذا البحث مناقشة الاحتياطات الواجب اتخاذها لمنع تلوث الألبان الخام للحفاظ عليها من الفساد وكذلك صحة المستهلك.

SUMMARY

Chemical and microbiological analysis were carried out on 75 raw milk samples randomly collected from some dairy farms of Assiut city to evaluate the sanitary condition of raw milk consumed in Assiut. The keeping quality tests which depend on increase amount of acidity revealed an average 6.8 with the pH value and 0.16% as acid percentage with the titrable acidity. Clot on boiling and Alcohol precipitation tests

scored 36 and 41.3% respectively. The results of keeping quality tests depend on increase amount of enzymes recorded 10 good samples (13.3%), 8 fair (24%), 21 bad (28%) and 26 very bad samples (34.7%), with resazurine test. The amount of free oxygen in the simple catalase tube test was 0-2 in 12 good samples (16%), 2-5 in 24 fair samples (32%) and was bad in 39 samples (52%) more than 5cc free O₂. In case of microbiological analysis the averages of total bacterial, coliform, yeast and mould counts were 4×10^6 , 9×10^3 , 12×10^3 and 3×10^3 /ml respectively. Examination for anaerobic spore-formers detected 25.3% of the examined samples. The public health importance of the counted organisms and the prophylactic measures to improve the quality of dairy farm milk were discussed.

Key words: Sanitary, Chemical, Microbiological, Analysis, raw milk.

INTRODUCTION

Milk is an important natural food and it exceeds all other foods in the variety and quality of contents with which milk furnishes the human body at all stages of life. So, Prophet Mohamed each time drinks milk calls to Allah to bless milk and to be increased more than other foods.

High quality raw milk free from pathogenic microorganisms, with less than 50,000 microorganisms/ml is a growing concern worldwide by the public health authorities, milk industries and consumers due to firstly, the production of high quality dairy products secondly to reduce the significant increase in the incidence of milkborne illnesses (Desmaures *et al.*, 1997).

Milk may be subjected to many risks of contamination from various sources from the time it leaves the udder until it is consumed or manufactured. Milk may become contaminated by small numbers of bacteria from a disease-free udder but the surrounding environment during milk handling and from milk equipments contribute in a high contamination (Thomas *et al.*, 1971 and Cousins and Bramley, 1981). Lack of cooling facilities and high atmospheric temperatures beside the poor hygiene during transportation attribute in lowering the raw milk quality. The raw milk microbial quality is of primary concern to throw light early on the udder which consequently affects the bulk milk appearance, odor, taste and dramatic changes in milk composition and production (Hogan and Smith, 1987 and Sargeant *et al.*, 2001). Several methodologies are used to evaluate the quality control of raw milk. The most important indices of microbiological quality are the total bacterial

count, coliforms, yeasts, moulds and detection of anaerobics poreformers as recorded by A.P.H.A. (1992). The aerobic plate count was determined by many workers as an index of hygienic measures and sanitary care of equipments, utensils and storage conditions on the farm during milking (Yadava *et al.*, 1985 and Kodikara, 1986). Thus, the dairy regulations in some twons include the total aerobic counts to grade milk for the producer quality-payment scheme.

Coliform as a Gram negative primary pathogen (Harmon, 1994) does not present in udder of dairy animal but in its surroundings (soil, bedding and manure). It is also a normal inhabitant of the intestinal tract of man and animals therefore, coliform count is a part of the microbiological criteria to indicate the hygienic status during milking. Hogan and Smith (1987), pointed out that more than 100 coliform cfu/ml indicates the bad hygiene during milking. According to the (W.H.O., 2000) coliforms are responsible for diarrheal disease of 30% in industrial countries and hundred of millions in developing countries. El-Kosi (2001) obtained higher incidence and counts of coliforms and fecal coliforms from raw milk products.

Fungi including yeasts and moulds are useful as a part of the microbiological analysis to indicate the hygienic status during milking, handling and the storage of milk. They can grow over a wide range of temperatures and gain entrance to raw milk from the contaminated air, dust and utensils. Presence of fungi is of great concern wheather from the public health or economic points of view. Molds are capable of producing toxic secondary metabolites known as mycotoxins causing cases of food poisoning and liver cancer in human (Mossel, 1982). Fungi have probably received more attention by the dairy industries owing to the undesirable changes and defects occurred in milk and milk products under adversity of pH, moisture and temperature leading to down grading of the product (Beuvier *et al.*, 1997). The presence of high counts of these organisms in raw milk is indicative of unsatisfactory sanitation during the milk journey from the time it elapses the udder till reaches to the consumer. Many trials by several examiners were paid to count yeasts and moulds from raw bulk milk (Sabry, 2001, Al-Ganzoury, 2002). Anaerobic sporeformers detection is of great concern to indicate the faecal pollution since, they are normal inhabitant of the lower part of the intestinal tract of worm blooded animals (Smith and Holdeman, 1981) and about 25% of the human populations excrete the bacteria in their feces (Gudkov and Dolidze, 1975). A complete relation between

the hygienic production of milk and the presence of the anaerobs. Milk and other dairy products implicated as a cause of *Clostridium perfringens* food poisoning (Woodward *et al.*, 1970 and Anon, 1982).

Keeping quality tests which include those depend on increase amount of acidity as pH values, titrable acidity, clot on boiling and alcohol precepitation tests as reported by Lampert (1984) that the acceptable level for titrable acidity of fresh raw milk is 0.12-0.16%. While, those depend on increase amount of enzymes as resazurine and catalase tests together with microbiological analysis were found to be reliable indices to evaluate the sanitary conditions under which milk have been produced and handled (Garvie and Rowlands, 1952; Nakae *et al.*, 1978; Yadava *et al.*, 1985 and Kodikara, 1986). Therefore, the production of high quality milk should be a priority and of great importance to the economy of the dairy farms and the sustainable development of the dairy industry.

A considerable amount of research has been devoted on the study of the sanitary condition of raw milk by many workers and several authors at different countries (El-Leboudy *et al.*, 1992; Harmon, 1994; El-Shinawy *et al.*, 1995; Desmasures *et al.*, 1997 and Sabry, 2001).

This preliminary study was planed to evaluate the sanitary condition of raw milk of some dairy farms in Assiut city by applying the following keeping quality tests:

- 1- Tests depend on increase amount of acidity including:
 - pH value
 - Titrable acidity
 - Clot on boiling and alcohol precipitation
- 2 – Tests depend on increase amount of enzymes including:
 - Resazurine
 - Simple catalase test
- 3 – Microbiological examination to determine:
 - Total bacterial counts
 - Coliform count
 - Yeast and mould counts
 - Detection of anaerobs.

MATERIAL and METHODS

Seventy five raw milk samples were collected randomly and aseptically in screw-cap bottles from some of Assiut city dairy farms. Samples were transferred to the laboratory in Harare as possible and

stored at 4°C until analysis that began within 24 hrs of sampling. Milk samples were tested by Storch test according to Lampert (1975) to exclude the heat treated milk samples.

Chemical analysis:

- 1- Keeping quality tests depend on increase amount of acidity:
 - pH value according to (APHA, 1992). Using electrical digital pH meter (an Orion Model).
 - Titrable acidity according to (Case *et al.*, 1985).
 - Clot on boiling and alcohol precipitation tests were applied according to (APHA, 1992).
- 2- Keeping quality tests depend on increase amount of enzymes adopted according to (APHA (1992) and Chalmers, 1992). Resazurine and Catalase tube test.

Microbiological analysis:

Total bacterial counts: by applying the standard plate count technique according to APHA (1992).

Coliform count: using Violet Red Bile agar (FAO, 1992) as recommended by Mercuri and Cox (1979).

Total yeasts and moulds count: according to Harrigan and McCance (1976) using Sabaroud Dextrose agar (Difco Lab.).

Anaerobic sporeformers: by stormy fermentation test according to Cruickshank *et al.* (1969).

RESULTS

The obtained results were tabulated in Tables 1-3.

Table 1: Statistical analytical results of keeping quality tests depend on increase amount of acidity of the examined raw milk samples.

Parameter	No. of examined samples	Min	Max.	Average	+ve samples above acceptable level (0.12-0.16%) Lampert, 1984	
					No/75	%
pH	75	6.7	6.9	6.8		
Titration acidity	75	0.12	0.18	0.16	39	52
					36	48
	No. of +ve/75				%	
Clot on boiling test		27		36		
Alcohol precipitation test		31		41.3		

Table 2: Statistical analytical results of keeping quality tests depend on increase amount of enzymes of raw milk samples.

Total No. of samples	Resaurine test				Simple catalase test			
	Grade	Color	No. of +ve	%	Grade	Amount of O ₂	No. of +ve	%
75	Good	Blue violet	10	13.3	Good	0-2	12	16
	Fair	Red violet	18	24	Fair	2-5	24	32
	Bad	Red	21	28	Bad	More than 5	39	52
	V. bad	Colorless	26	34.7				

Table 3: Statistical analytical results of different types of organism counts of raw milk samples.

Type of organism	No. of +ve samples/75	%	Min.	Max.	Average of +ve samples	+ve samples above the EOS (2001)	
						No.	%
T.C.C.	75	100	1x10 ⁴	2.8x10 ⁸	4x10 ⁶	28	37.3
Coliform	31	41.3	>x10 ²	2x10 ⁵	9x10 ³	24	32
Yeast	61	81.3	>10 ²	8x10 ⁵	12x10 ³	-	-
Mould	49	65.3	>10 ²	8x10 ⁴	3x10 ³	-	-
Anaerobic spores	19	25.3	-	-	-	-	-

DISCUSSION

A wide spread concern with increasing focus on the milk quality on dairy farms and dairy industries was noticed in recent years to safeguard consumers and to be a priority of establishment of strong market for milk and milk products.

Although the quality control tests measured the activity of organisms present in the examined samples in Table 1 showed that the average value of pH and the acidity percentage by titrable acidity was 6.8% and 0.16% respectively, 39 (52%) and 36 (48%) of the examined samples were above the acceptable limit recorded by Lampert (1984) and consequently they were unfit for heat treatment, processing and human consumption. El-Leboudy *et al.* (1992) and Sabry (2001) scored lower results than that obtained, while higher values were obtained by Mutukumira *et al.* (1996). Determination of pH value is an effective predictor of instability in the end product and is the most useful made on the milk after bulk storage as it indicates its real acidity.

Although titrable acidity expressed as lactic acid in milk. Al-Ashmawy (1990) preferred the pH value than the titrable acidity and considered it as less variable. Following up the keeping quality tests depending on increase amount of acidity, Table 1 indicated that 27 (36%) and 31 (41.3%) of the samples were sour and unfit for heat treatment with clot on boiling and alcohol precipitation tests, respectively. Alcohol precipitation test is more sensitive than clot on boiling test since it gives positive results with colostrum, mastitic milk due to *E. coli* and sweet curdling due to rennin like enzyme produced by the aerobic spore formers. It is useful for determination of the stability of milk to evaporation and sterilization by heat. It was observed during this study that the pH values and the concentration of titrable acidity of some of the samples suggested that the raw milk had started souring when samples were collected hence, milk was subjected to high temperatures which may have led to rapid multiplication of microorganisms resulting in souring of the milk samples.

In Table 2 resazurine test which is based on the reduction of a dye to a colorless compound by metabolic activities of bacteria indicated that 13.3, 24, 28 and 34.7% of the samples graded good, fair, bad and very bad, respectively. The results were not in accordance with Sabry (2001) and Al-Ganzoury (2002). They were in agreement with El-Leboudy *et al.* (1992) who graded 58.3 and 52.5% of inferior quality cow's and buffalo's milk samples with Methylene blue reduction test, respectively. Reduction tests are considered by several authors as indicative for the sanitary conditions under which milks were produced Garvie and Rowlands (1952), while, Resazurine test is considered a rapid and simple method for grading milk.

Results of simple catalase tube test in Table 2 revealed that 39 (52%) of the examined samples were of bad quality and should be rejected as the amount of free oxygen was more than 5CC and the results were disagreed with Al-Ganzoury (2002). Only, 12 (16%) and 24 (32%) of the samples graded good and fair, respectively. Catalase tests depend on the amount of catalase enzyme, which present normally in milk but in little amount. So, they are used to detect the abnormal milk based upon somatic cell contents which accelerate the decomposition of H_2O_2 by catalase enzyme.

Concerning the microbiological analysis, Table 3 cleared that the raw milk samples examined were positive for the standard plate (100%) and the total aerobic counts ranged between 1×10^4 and 2.8×10^8 cfu/ml

with an average 4×10^6 . Nearly similar results were obtained by El-Leboudy *et al.* (1992), El-Shinawy *et al.* (1995), Mutukumira *et al.* (1996) and Abdel Moghney *et al.* (2001). The results were higher than Desmaures *et al.* (1997) and Al-Ganzoury (2002). High total aerobic count is usually used to assess the overall sanitation and storage condition of the raw milk. Marshall (1982) and Muir *et al.* (1986) reported that the high total aerobic count not only lead to bad quality of raw milk but also quality defect in milk products.

According to the Egyptian Standard (EOS, 2001) and the British one (Thomas and Thomas, 1975) which pointed out that more than 2×10^5 bacteria/ml have poor quality it was obvious that 28 (37.3%) of the examined samples were above the Egyptian and the British Standards. Therefore, the obtained results of the examined dairy farm milk samples were of poor quality and that reflects the sanitary conditions adopted on the farms during milking, handling, storage and transportation. The variation in results of the total bacterial counts may be linked to the alternative use of alkaline and acid detergents during milking process (Chatelin and Richard, 1983). The giving results in Table 3 showed that 31 (41.3%) of the examined raw dairy farm milks have an average count of coliform 9×10^3 /ml. Different results obtained by the same above mentioned specialists. According to the EOS (2001) which pointed out that the limited count by coliform was 100 coliform cells and less than 1000 cells/ml of milk, the result obtained included 24 (32%) samples were above the limited Egyptian Standard and only 5 samples were within the limited standard. Therefore, presence of large numbers of coliform bacteria in raw milk provides an index of the hygienic standards used during the production of milk. Public health concern intended for coliform especially in children who subjected to certain cases of gastroenteritis when intended for liquid utilization of not heat treated milk (Cruickshank *et al.*, 1984). Coliforms incriminated in food poisoning and cases of cystitis and pyelonephritis (Finegold and Martin, 1982). Approximately 70 to 80% of coliform infections give clinical or systemic symptoms of mastitis (Harmon, 1994). Coliforms also have economic importance for the disgrading of the milk value affecting the economic condition of the dairy plant through milk and milk products spoilage including acid production, sliminess, ropiness, bitter and soapy flavours, grassy unclean fecal odor (Stead, 1986). During the course of the microbiological analysis of the samples yeasts and moulds were predominant. They revealed 61 (81.3) and 49 (65.3%)

with averages 12×10^3 and 3×10^3 /ml respectively. The results did not agree the findings by several examiners who could not detect yeasts and moulds from raw bulk milk (Hussain *et al.*, 1984; El-Rashidy, 1986; El-Kholy *et al.*, 1994 and Mutukumira *et al.*, 1996). The results were higher than that obtained by Desmases *et al.*, 1997 and Al-Ganzoury, 2002. Presence of yeasts and moulds in raw milk is indicative for the neglected sanitation during the milking process. Presence of yeasts and moulds in raw milk is of great concern not only to the health of human and animals due to the mycotoxins production which responsible for food poisoning and liver cancer in human (Mossel, 1982), but also they can cause lipolytic and proteolytic spoilage which influence the biochemical characters and flavour of the product and commercially be undesirable and down grading (Beuvier *et al.*, 1997).

From Table 3 the anaerobic sporeformers bacteria revealed 19 (25.3%). They are higher than that obtained by Abdel-Hakeim (1992) and some what near to Desmases *et al.* (1997). Other investigators could isolate the organism from milk and other milk products El-Bassiony (1980) and Saad (1995). The presence of the anaerobic bacteria in milk is indicative for the neglected sanitary measures during production and distribution since the organism is more widely distributed than any other pathogenic bacteria occurring in soil, dust and among the intestinal microflora of warm-blooded animals (Smith and Holdeman, 1981) and so, it is considered as one of faecal contaminants (Romagnoli and Brazzi, 1960). Anaerobic sporeforms bacteria have the ability to release toxin in the alimentary canal after ingestion of contaminated food (8-15 hs) causing foodborne illness 15 h, also they are accepted as an etiological agent of gas gangrene in man and animals (Hobbs, 1965) and acute mastitis (Johnston, 1986). Milk and other dairy products incriminated as a cause of clostridium food poisoning (Woodward, 1970 and Anon, 1982)

It is worth to mention that considerable variations in the microbial content of the raw examined dairy farm milk samples were noticed during the microbiological analysis and this may be attributed to the hygienic standard during milking and storage facilities adopted in each dairy farm. It was obvious that the most raw milk contaminations were yeasts than bacteria to ensure the neglected sanitation adopted.

The microbiological, and chemical properties of raw milk are the basis for the high quality milk and milk products. Therefore, sanitary control measures including the hygienic condition of the animal, milkers

and equipments used for milking should be adopted during production, handling and storage of milk, beside the applying of the quality control tests from time to time to grade dairy farm milks.

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