IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 12 Ver. II (December. 2016), PP 59-64
www.iosrjournals.org

Evaluation of Raw Milk from Local Markets and Milk Samples Taken Directly From Cows in Basrah-Iraq.

Ali A. Al-Iedani[1] and Samar S. Ghazi[2]
[1,2](Department of Microbiology and Parasitology/ College of Veterinary Medicine/ University of Basrah/ Iraq)

Abstract: *The present study aimed to compare bacteriologically, chemically and physically between raw milk samples collected from local markets of Basrah, Iraq and samples collected directly from apparently normal cows, it also aimed to study the effects of subclinical mastitis on the quality of milk. A total of (205) samples were collected including: 100 samples from cows (direct samples) and 105 from local markets (indirect samples). By using California mastitis test (CMT), 38% of direct samples were positive (direct positive).The samples collected from local markets when compared with direct negative samples (direct negative) characterized by high values of total bacterial count, total coliform count, freezing point, pH and titratable acidity. However, low values were recorded for other indicators including: fat, solid not fat (SNF), lactose, protein and relative density. Test of difference between direct negative and indirect samples by using two tailed T test revealed following: highly significant difference was detected for total bacterial count, fat, SNF, lactose, protein, freezing point, pH, and titratable acidity, whereas, significant result was for total coliform count. On the other hand, the difference for relative density was not significant.Regarding the direct positive samples when compared with direct negative samples characterized by high values of total bacterial count, total coliform count, fat, protein, relative density and pH. Whereas, low values were recorded for SNF, lactose, freezing point and titratable acidity. The difference between direct negative and direct positive samples by using two tailed T test revealed following: highly significant difference was detected for titratable acidity, significant result was for total coliform count and relative density, however, the difference for total bacterial count, fat, SNF, lactose, protein, freezing point and pH was not significant. In conclusion, the indirect samples, high bacterial and coliform counts indicated that the handling of milk from the collection until reaching the consumer was unhygienic. However, the low percentage of fat, SNF, lactose, protein and relative density, this probably referred to adulteration of milk by water. The higher pH value may be result from using materials decrease the acidity of milk. Regarding the direct samples 38% of tested milk samples were positive for California mastitis test, the changes in affected samples (direct positive) were not considerable, ultimately the elevation of bacterial count especially coliform may constitute a high degree of danger to public health.*

Keywords: *Raw milk, Subclinical mastitis, Total bacterial count, Total coliform count*

I. Introduction

Milk is an important source of nutrients to human and animals. It is mean to be the first and the only food for the offspring of mammals as is nearly complete food (1). Milk has a complex biochemical constituent and its high water activity and nutritional value serves as a good medium for growth and multiplication of many types of microorganisms when suitable conditions exists (2).The number and types of micro-organisms in milk immediately after milking are influenced by factors such as animal and equipment cleanliness, season, feed and animal health (3). Bacterial contamination of raw milk can originate from various sources: air, milking equipment, feed, soil, feces and grass (4). Spoilage microorganisms include aerobic psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli and spore-forming bacteria (5).Mastitis can manifest itself in either clinical or subclinical form. Subclinical mastitis occurs when both milk and mammary gland appear normal but Somatic Cell Counts (SCC) are elevated to a level above 200,000 cells/mL (6). It is causes change in the milk composition and any change in its percentage in turn affect the suitability of milk processing and quality of its product (7), degree of these changes depends on the infecting agent and the inflammatory response (8). It is causes lowers of the hygienic value of milk, reduce milk production in addition to treatment coast (9).Adulteration is defined as the process which the quality or the nature of substance is reduced, it is maybe intentional or unintentional (10). Water is the most common adulterant (11).This study aimed to: compare bacteriologically, chemically and physically between raw milk samples collected from local markets and samples collected directly from apparently normal cows, and to study the effects of subclinical mastitis on the quality of milk.

II. Material and methods

This study was conducted from October (2015) to March (2016), the total number of milk samples were (205) included (100) milk samples from normally apparent cows (direct samples) and (105) milk samples from local markets (indirect samples). Direct samples were tested by California Mastitis Test (CMT). The test was done according to (6).

Bacteriological methods

Total bacterial count was done by pour plating methods. The appropriate dilution of milk samples (10^{-1},10^{-2},10^{-3}) were selected, one ml from milk dilution was transferred with sterile pipette to a petri dish and 15 ml of nutrient agar was added at (48°C), the medium was allowed to solidify, the plates were incubated at 35°C for 48hrs (12). Total coliform was done by added one ml aliquot of milk dilution to a petri dish and 10 ml of Violet Red Bile Agar at (48°C) was added. The medium was allowed to solidify before incubating at 32 °C for 18-24 hrs. The plate was examined for purple-red colonies (12).

Physical and chemical analysis of milk samples

1-pH: The pH of milk samples was determined in the laboratory using a digital pH-meter based on the procedure described by (13).

2-Titratable acidity of milk: Titratable acidity of the milk samples was determined according to the method of (14).

3-Instrumental analysis: Lactoflash system was used for rapid analysis of the main constituents of milk. It directly measures fat and SNF and then calculates density, protein, lactose and freezing point. Sample capacity about 10 to 12 ml of milk (15).

III. Results

Samples

The total number of milk samples collected from apparently normal cows was 100, also 105 samples was collected from local market. Samples which collected from cows were subjected to indirect mastitis test, 38% of samples were positive for CMT.

Total bacterial and Coliform count

The results in Table (1) revealed that the values of total bacterial count were the highest (6.42±6.68) from indirect samples and the lowest values (4.08±4.31) from direct negative samples. Measuring the difference of means by using two tailed T test revealed that the difference between direct negative and indirect samples was statistically highly significant ($p<0.001$), whereas, the difference between negative and positive direct samples was not significant ($P>0.05$).The highest values of total coliform count explicated in Table (1) were (6.28±6.73) from direct positive samples, the lowest values (2.225±2.155) from direct negative samples. Two tailed T test result indicated that the difference between direct positive and indirect was statistically significant ($p<0.05$), also, the difference between negative and positive direct samples was also significant ($p<0.05$).

Table (1): Total bacterial and coliform count of direct and indirect milk samples.

Samples	Total bacterial count Mean ± SD $\log^{10}$	Total Coliform count Mean ± SD $\log^{10}$
Direct negative	4.08 ± 4.31	2.225 ± 2.155
Direct positive	4.28 ± 4.71	6.34 ± 6.812
Indirect	6.4 2± 6.68	6.287 ± 6.737

Direct negative = samples collected from cows apparently normal and negative for CMT.
Direct positive = samples collected from cows apparently normal and positive for CMT.
Indirect samples = samples collected from local markets.

Chemical analysis of samples

Results of Table (2) showed that the values of fat were the highest (3.9989±1.21822) in the affected samples (direct positive) and the lowest values were recoded from indirect samples (2.2865±0.80888). Compare of means by using two tailed T test revealed that the difference between direct negative and indirect was statistically highly significant ($P<0.001$). However, the difference between positive and negative samples was statistically not significant.The values of SNF were the highest (7.8567±1.13051) in the normal samples (direct negative) and the lowest values were revealed from indirect samples (6.3618±1.39700). Compare of means by using two tailed T test revealed that the difference between direct negative and indirect was statistically highly significant ($P<0.001$), whereas, for direct positive samples was not significant. Table (2) showed that the values of lactose were the highest (4.6142±0.65846) in the samples (direct negative) and the lowest values were showed from indirect samples (3.1379±0.82834). The difference by using tow tailed T test (compare of means) between direct negative and indirect was statistically highly significant ($P<0.001$), on the other hand, the

difference between direct samples was not significant.Regarding protein percent Table (2) showed that the values of protein were the highest (3.0609±0.55580) in the samples (direct positive) and the lowest values were recoded from indirect samples (2.2365±0.64248). Measuring the difference between means by using T test explicated that the difference between direct negative and indirect was statistically highly significant (P<0.001). However, the difference between direct negative and direct positive was not significant (P>0.05).

Table (2): Chemical analysis of direct and indirect milk samples.

Samples	Fat Mean ± SD	SNF Mean ± SD	Lactose Mean ± SD	Protein Mean ± SD
Direct negative	3.7029 ± 0.86080	7.8567 ± 1.13051	4.6142 ± 0.65846	3.0398 ± 0.56347
Direct positive	3.9989 ±1.21822	7.854 2±1.21780	4.4963 ± 0.60960	3.0609 ± 0.55580
Indirect	2.2865 ± 0.80888	6.3618 ±1.39700	3.1379 ± 0.82834	2.2365 ± 0.64248

Physical analysis of samples

Physical analysis of milk samples indicated that the relative density value of affected samples (direct positive) was the highest (1.1984±0.20005) however, the lowest value was from indirect samples (1.0306±0.00817). Tow tailed T test result indicated that the difference between direct negative and indirect samples was statistically not significant (P>0.05), however, the difference between direct samples was significant (P <0.05).According to the Table (3) which revealed that the values of freezing point were the lowest value (-0.53839 ± 0.056668) in the affected samples (direct positive) and the highest value (0.46104±0.089498) obtained from indirect samples. Two tailed T test revealed that the difference between direct negative and indirect was highly significant statistically (P<0.001).The Table (3) explicated the values of pH were the highest (7.101479 ± 0.2640885) in the indirect samples and the lowest values were explicated from direct negative samples (6.588274±0.4822700). Two tailed T test revealed that the difference between direct negative and indirect samples was highly significant statistically (P<0.001), however the difference between direct positive and direct negative was not significant (P>0.05). Physical analysis of milk samples showed that the titratable acidity of indirect samples was the highest (0.0212892±0.00809420) and the lowest values were from direct positive samples (0.009174±0.00022540). Compare of means by using two tailed T test explicated that the difference between direct negative and direct positive was very significant (P<0.001), however, the difference between direct negative and indirect samples was not significant (P>0.05).

Table (3): Physical analysis for direct and indirect milk samples.

Samples	Relative density Mean ± SD	Freezing point Mean ± SD	pH Mean ± SD	Titratable acidity Mean ± SD
Direct negative	1.0322 ± 0.03196	-0.52642±0.74761	6.588274±0.4822700	0.014319±0.0018717
Direct positive	1.1984 ± 0.20005	-0.53839±0.056668	6.993158±0.2684507	0.009174±0.0022540
Indirect	1.0106 ± 0.00817	-0.46104±0.089498	7.101479±0.2640885	0.021289±0.00809420

IV. Discussion

Samples collection and result of California Mastitis Test:

Subclinical mastitis is one of the most prevalent, important and costly diseases of dairy animals worldwide, with losses of over 1.7 billion dollars a year in the USA alone (16). The different rates of subclinical mastitis in different countries may be due to the difference in animals breed, management conditions and methods of diagnosis (17).Result of this study indicate that 38% of milk samples was positive with California Mastitis Test (CMT), this result is in agreement with (18).

Total bacterial and total coliform count

In this study the direct samples (CMT positive) showed high level of total bacterial count when compared with direct negative, this result agreed with (19), who indicated that the infected quarters by a major pathogen gave rise to 46.6% of the total number of colony forming unit. (20) stated that the total number of bacteria in raw milk should not exceed more than (5.698) $\log^{10}$ cfu per 1ml milk.The total bacterial count of indirect samples was the highest (6.42±6.68) and this result is in accordance with (21) and (22) who concluded that the market milk samples had higher bacterial count. Microorganisms were introduced to milk by a number of ways, such as, excretion from the udder of infected animals or contamination from dairy farm environment, packaging, and production facilitates (23). Coliforms are almost always found in raw milk but with good methods of production number of coliforms can be kept very low (24). The presence of these organisms in milk and milk products is an indication of unsanitary production and/or improper handling of either milk or milk utensils (25). In this study the value of total coliform count was high in milk samples positive for CMT. These results are in agreement with (26) and (27) who reported that the milking udder with sub-clinical mastitis and wet environment lead to contamination of raw milk and reaches the consumers with elevated coliform count. Moreover, the count of coliform was high in samples collected from local markets, this result is in accordance

with (28) who noted that coliform count above 500 cfu/ml indicates poor hygiene either during equipment cleaning or between milking with common contaminants such as bedding, manure, water.

Chemical analysis of samples

The percentage of fat in cows which affected with subclinical mastitis (direct samples) was increased about (3.9989±1.21822) this results were in agreement with (29) who reported that milk fat increased during subclinical mastitis. According to (30) the increase in fat concentration indicates that there is a reduce in lactose synthesis and reduced milk volume. The difference between direct negative and indirect highly significant, the lowest fat percentage of milk from indirect samples may be due to the adulteration of milk by added water or skimming, this result is in agreement with (31). The percentage of SNF in direct positive samples was (7.8567±1.13051), whereas lower percentage was from milk samples infected with sub-clinical mastitis. The decrease of SNF in direct positive samples may be caused by infecting pathogen which reduce the synthetic activity of mammary gland, this result is in agreement with (32). The difference between direct negative and indirect was highly significant ($p<0.001$), this result is in agreement with (33), (34), (35), (36), (37) and (31) who noted that the milk sold at market was lower due to malpractices such as skimming and adulteration with water.The values of lactose in Table (2) of direct samples (CMT positive) were lower than direct samples (CMT negative) these results are in agreement with (38) who noted that mastitis causes decrease in milk lactose through damaging the secretory cells that produce milk in mammary gland. The lactose content in indirect samples also was low due to adulteration of milk by added water, this result is in agreement with (39). The protein percent was the highest in positive samples due to affection with subclinical mastitis and this result is in agreement with (40) who showed that the protein in milk sample from affected cows with SCM increased. (41) reported that the increment in protein concentration is caused by alteration in the permeability of the secretory epithelium and capillary wall, these changes induced by bacterial toxins. Also the difference between direct negative and indirect was highly significant due to commercial adulteration by added water to milk this result is in accordance with (42), (43), (37) and (44).

Physical analysis of samples

The density of milk at 20°C should be within the range of 1.028-1.036 g/ml. The difference between direct negative and direct positive was very significant due to descent the inflammatory cells with milk, this result is in accordance with (40) and (49) who showed increase of protein concentration in affected milk samples increased and increase amounts of sodium and chloride. While density of indirect milk samples lower due to commercial adulteration by added water this result is in agreement with (45), (44) and (46) who noted that adulteration of extraneous water in milk apparently increase the moisture content of corresponding milk, so this lead to lower the relative density.The freezing point of milk is an important indicator of the milk quality. Increment of freezing point of indirect samples (samples that taken from market) are in agreement with (39) who noted that added extraneous water to milk samples increase the freezing point. While the freezing point of affected samples was the lowest maybe due to descent of solutes from blood to the milk during inflammation of udder. The normal pH for raw milk is about 6.6 (47). The pH of affected samples increased (6.993158±0.2684507) these results are in accordance with (37) who reported that the difference between affected and not affected was highly significant. The pH value for indirect samples was (7.101479±0.2640885) in accordance with (48) who noted that the use neutralizers such as (caustic soda, caustic potash sodium carbonate, sodium bicarbonate and lime water) resulting in neutral or basic pH, in spite of growth and multiplication of bacteria.

Normal fresh milk has apparent acidity range from 0.014% to 0.016% (13). Table (3) showed that the titratable acidity for direct positive samples was the lowest, this result is in agreement with (49) who reported that the change of ionic equilibrium often due to increase the amounts of sodium and chloride and reduced potassium ion in mastitic milk as the most important reason. The titratable acidity of milk samples obtained from markets were recorded higher values this result is in agreement with (50) who recorded high titratable acidity value from market milk samples and might be due to bacterial growth and multiplication during transportation of milk and longer storage of milk before sale.

V. Conclusion

The results of this study revealed that 38% of milk samples were positive for subclinical mastitis (CMT test). The percentage of total bacterial count and total coliform count in indirect milk samples were elevated. These results of high bacterial count in milk samples collected from local markets indicated that the handling of milk from the collection until reaching the consumer was unhygienic.With regard to commercial adulteration, the study found that the percentage of fat, SNF, protein, lactose and relative density were low in indirect milk samples, this probably referred to cheating of milk with water.

The pH value for normal direct samples were very close to normal value, while direct samples which were positive for CMT had high pH value due to affection with mastitis. Values of indirect samples were the highest and this may be result from using materials that decrease acidity of milk.Concerning titratable acidity, it was almost normal in direct samples negative for CMT, whereas, direct positive samples had lower values than normal and the reason may be due to descent some ions. However, the titratable acidity of indirect samples was high due to presence lactic acid bacteria and also bad storage that help the growth of this bacteria.

References

[1] Pandey, G. and Voskuil, G. (2011). *Manual on Milk safety, quality and hygiene.* Golden Valley Agricultural Research Trust, Zambia.

[2] Parekh, T. and Subhash, R. (2008). Molecular and bacteriological examination of milk from different milk animals with special reference to Coliforms. *Current Res. in Bacteriol.; 1(2):* 56 - 63.

[3] Rogelj, I. (2003). *Microbiology of food of animal origin*. Ljubljana. Biotechnology University, section of food; 515–538.

[4] Coorevits, A.; De Jonghe, V.; Vandroemme, J.; Reekmans, R.; Heyrman, J.; Messens, W.; De Vos, P. and Heyndrickx, M. (2008). Comparative analysis of the diversity of aerobic-spore-forming bacteria in raw milk from organic and conventional dairy farms. *Syst. Appl. Microbiol.; 31:*126-140.

[5] Entis, P.; Fung, D.; Griffiths, M.; McIntyre, L.; Russell, S.; Sharpe, A. and Tortorello, M. (2003). Rapid methods for detection, identification, and enumeration. In Downes, F. P., & Ito, K. (eds.) Compendium of methods for the microbiological examination of foods 4th ed. Washington, DC: Am. Public Health Association.

[6] **Michael, M. (2011).** California Mastitis Test and Milk Quality. *Michigan Dairy Review; 16(2)*:1-3.

[7] Vivar-Quintana, A.; Beneitez Delamano, E. and Rrvilla, I. (2006). Relationship between somatic cell count and the properties yoghurt made from ewe's milk. *Int. Dairy. J.; 16*:262-267.

[8] Sharif, A. and Muhammad, G. (2009). Mastitis control in dairy animals. *Pak. Vet. J.; 29*:145-148.

[9] AL-Majali, A. and Jawabreh, S. (2003). Period prevalence and etiology of subclinical mastitis in Awassi sheep in southern Jordan. *Small Ruminant Res., 47:*243-248.

[10] Kumar, M.; Rao, Y. And Gupta, M. (1981). Chemical Quality of Milk Based Sweets Sold in Agra and Mathura Cities. *J. Agr. Sci.; 23*:13-17**.**

[11] Chakravorty, S. and Chakravarty, A. (2011). An Investigation of adulteration in milk obtained from different localities of Varanasi city, *The Ind. J. Res. Anvikshiki*; *5:* 120-123.

[12] Marshall, R. (1993). *Standard methods for the examination of dairy products*, 16th ed. American Public Health Association, Washington, D.C.

[13] O'Connor, C. (1995). *Rural Dairy Technology ILRI Training Manual I*, International Livestock Research Institute, Addis Ababa, Ethiopia.

[14] AOAC (Association of Official Analytical Chemists) (1990). *Official Methods of Analysis*.15th ed. Association of Official Analytical Chemists. Washington, D.C.WWW.funke-gerber.de.

[15] Sahoo, N.; Kumar, P.; Bhusan, B.; Bhattacharya,T.; Dayal, S. and Sahoo, M. (2012). Lysozyme in livestock: a guide to selection for disease resistance: a review. *J. Animal Sci. Adv.; 2:*347-360.

[16] Anakalo, S.; Ogollah, H. and Nanua, J. (2005). Effect of subclinical mastitis on milk composition in the Kenyan smallholder. *African Crop Science Conference Proceedings*; 7: 545-550.

[17] Abera, M.; Demei, B.; Aragaw, K.; Regassa, F. and Regassa, A. (2010). Isolation and identification of *Staphylococcus aureus* from bovine mastitis milk and their drug resistance patterns in Adama town, Ethiopia. *J. Vet. Med. Anim. Health.; 2*: 29-34.

[18] Olde Riekerink, R.; Barkema, H.; Veenstra, S.; Poole, D.; Dingwell, R.T. and Keefe, G. (2006). Prevalence of contagious mastitis pathogens in bulk tank milk in Prince Edward Island. *Can. Vet. J.; 47*: 567–572.

[19] William, E. and Paul, M. (1980). *Dairy Cattle Feeding and Management* .6th ed. Wiley. USA

[20] Hassan, G.M.; Meshref, A.M.S. and Gomaa, S.M. (2015). Microbiological quality and safety of fluid milk marketed in Cairo and Giza governorates. *Curr. Res. Dairy Sci., 7*: 18-25.

[21] EEC. (Council Directives 92/46 EEC). (1992). Laying dowen the health rules for the production and placing on the market of raw milk, heat-treated milk and milk –based product. *Official Journal of Europe Communit., 268*:1-441.

[22] Vissers, M. and Driehuis, F. (2009). On-Farm Hygienic Milk Production. In: *Milk Processing and Quality Management*, Tamime, A.Y. (Ed). Blackwell Publishing, USA., pp:1-22.

[23] Boor, K.; Brown, D.; Murphy, S. and Bandler, D. (1998). Microbial and chemical quality of raw milk in New York State, *J. Dairy Sci.; 81*:1743-1748.

[24] EL-zubeir, I. and Ahmed, M. (2007). The hygienic quality of raw milk produced by some dairy farms in Khartoum-Sudan. *J. Microbiol.; 2*: 988-991.

[25] FAO (2008). Milk hygiene in *milking, milk production hygiene and udder health*. FAO Animal Production and Health Papers-78. FAO Corporate Document Repository. (CDR), pp. 1-7.

[26] Zadoks, R.; Gillespie, B.; Barkema, H.; Sampimon, O.; Oliver P. and Schukken, Y. (2007). Comparison of the Etiology of Environmental Mastitis in two herds of Dairy cows. *Slovak J. Anim. Sci.; 40(3)*: 132- 140.

[27] Murphy, S. and Boor, K. (2003). *Basic dairy Bacteriology*. Microbiological quality defects in fluid milk products: The evaluation of shelf life. Cornell University, Ithaca, New York.

[28] Kitchen, B. (1981). Review of the progress of dairy Science: milk compositional changes and related diagnostic tests. *J. Dairy Res. ;65*: 93-100.

[29] Bruckmaier, R. and Blum, J. (2004). Fractionized milk composition in dairy cows with subclinical mastitis. *Vet. Med. Czech; 8*:283-290.

[30] Lateef, M.; Faraz, A.; Mustafa, M.; Akhtar, P. and Bashir, M. (2009). Detection of adulterants and chemical composition of milk supplied to canteens of various hospitals in Faisalabad city. *Pak. J. Nutr.; 9*:139-142.

[31] Benchedly, H.; Boutinaud, M.; Bernierdodier, P.; Marnet, P. and Lacasse, P. (2009). Disruption of cell junctions induces apoptosis and reduces synthetic activity in lactating goat mammary gland. *J. Diary Sci.; 93*:2938-2951.

[32] Mustafa, M. (1990). *Chemical and hygienic quality of milk supplied to canteens of various hospitals and educational institutions in Faisalabad city*. M. Sc. (Hons) LM, Thesis, University of Agriculture, Faisalabad.

[33] Khan, B.; Mustafa, M.; Abdullah, M. and Yaqoob, M. (1991). Chemical and hygienic quality of milk supplied to canteens of various hospitals in Faisalabad city. *Pak. J. Agri. Sci.; 28 (4)*: 404- 407.

[34] Mustafa, M.; Khan, B.; Abdullah, M. and Khan, L. (1991). Chemical and hygienic quality of milk supplied to canteens of various educational institutions in Faisalabad city. *Pak. J. Agri. Sci; 28 (1)*: 9-12.
[35] Khan, M.; Rajah, K. and Haines, M. (1999). Quantitative techniques in the measurement of milk adulteration in Peshawar. *Pakistan. Intern. J. dairy technol.; 52(1):*20-25.
[36] Javaid, S.; Gadahi J.; Khaskeli, M.; Bhutto, M.; Kumbher, S. and Panhwar, A. (2009). Physical and chemical quality of market milk sold at Tandojam, Pakistan. *Pak. Vet. J.; l: 29 (1):* 27-31.
[37] Yarabbi, H.; Mortazavi, A.; Mehraban, M. and Sepehri, N. (2014). Effect of somatic cell on the physico–chemical and microbial properties of raw milk in different seasons. *IJPAES; 4(3)*: 289-298.
[38] Soomro, A.; Khaskhel, M.; Awais, M.; Shabir, G.; Ul haq, I.; Nawaz, S.; Ali, I.; Murtaza, G. and Nawaz, R. (2014). Study on adulteration and composition of milk sold at badin. *IJRANSS; 2(9*):57-66.
[39] Al-Iedani, A. (2016). Isolation of *Staphylococcus aureus* and coagulase negative *Staphylococci* form bovine subclinical mastitis and their impact on the chemical components of milk. *Bas. J. Vet. Res; 15(2):*153-163.
[40] Schultz, L.H. (1977). Somatic cells in milk – physiological aspects and relationship to amount and composition of milk. *J. Food Prot.; 40*:125.
[41] Khan, M.; Zinnah, M.; Siddique, M.; Rashid, M.; Islam, M. and Choudhury, K. (2008). Physical and microbial qualities of raw milk collected from Bangladesh agricultural university, dairy farm and the surrounding villages. *Bangl. J. Vet. Med.; 6 (2)*: 217-221.
[42] Ayub, M.; Ahmad, Q.; Abbas, M.; Qazi, I. and Hattak, I. (2007). Composition and adulteration analysis of milk samples. *Sarhad J. Agri.; 23 (4):* 1127-1130.
[43] Hossain, T.; Alam, K. and Sidkar, D. (2010). Chemical and Microbiological Assessment of Raw Milk and Processing Liquid Market Milks of Bangladesh. *Res. J. Dairy Sci.; 4 (4)*:28- 34.
[44] Paradkar, M.; Singhal, R. and Kulkarni, P. (2000). An approach to the detection of synthetic milk in dairy milk: 1. Detection of urea. *Int. J. Dairy Technol.; 53*: 3:87-91.
[45] Mansour, A.; El-Loly, M. and Ahmed, R. (2012). A Preliminary Detection of Physical and Chemical Properties, Inhibitory Substances and Preservatives in Raw Milk. *Internet J. Food Safety; 14:* 93-103.
[46] Webb, B.; Johnson, A.; Alford, J. (1974). *Fundamental of Dairy Chemistry*, 2nd Ed. Chapter I, AVI Publishing Co., Westport, CT.
[47] Miralles, B.; Bartlome, B.; Amigo, L. and Ramos, M. (2000).Comparison of three methods determine the whey protein to total protein ratio in milk. *J. dairy Sci.; 83(12):*2759-2765.
[48] Early, R. (1998). *The Technology of Dairy Products.* Thomson Science. London. U.K.; 1-25.
[49] Gemechu, T.; Beyene, F. and Eshetu, M. (2015). Physical and Chemical quality of raw cow's milk produced and marketed in Shashemene town, southern Ethiopia. *J. Food and Agri. Sci.;5(2):*7-13.