Detection of Kids milk Quality using Methylene Blue Reduction test

Sewgil S. Anwer[1], Sahar Muhammed Zaki[2], Sarween A. Rasul, Ronar J. Hassan[3], Iman J. Ahmad[4], Attia J. Qader[5]

[1,3,4,5] Department of Clinical Biochemistry, College of Health Science, Erbil, Iraq
[2] Department of Medical Microbiology, College of Health Science, Erbil, Iraq

Abstract— Back ground and Objectives: Milk is a highly nutritious food that serves as an excellent growth medium for a wide range of microorganisms. Rapid, simple and inexpensive microbiological quality determination methods including Methylene Blue Reduction (MBRT) test could be commonly used as a quick method to assess the microbiological quality of raw and pasteurized milk. The aim of study is to determine quality of kids milk using Methylene Blue Dye Reduction Test

Methods: A total of 37 samples comprising of kids milk collected at different levels of collection and processed. Accordingly 12 different milk samples from hypermarket, 8 different milk samples from unlicensed hawker (retail market), 11 different samples with additives from hyper market samples and 6 different samples with high price. Samples were collected. One ml of the Methylene Blue Thiocyanate solution added into a test tube then 10 ml of milk poured into test tube. Tubes incubated at 37 °C

Results: Results showed that all types of milk that purchased from super market, local market and high price milk types shown no change of methylene Blue color appear on the base of time, that indicate very good quality of the milk. On the base of milk types with additive materials only one milk showed change in colour but after confirm test the colour remained blue and not changed.

Conclusion: Methylene blue reduction test is rapid economic method that can be used for detection of milk quality. Approximately all the kids of milks that is purchased in our market and local markets showed sterility and the source contamination if take place may be by storage condition and transvers vehicle.

Keywords— kids milk, Methylene blue reduction test, decoulorization.

I. INTRODUCTION

Milk is the nutrient rich liquid secreted by the mammary gland of mammals. It provide the primary sources of nutrition for newborns before they are able to digest other type of food the early lactation of milk is known as colostrum's and carries the mother antibodies to the baby, it can reduce the risk of many diseases of the offspring. [1,4,5]

Good quality milk should have a pleasantly sweet and clean flavor with no distinct after taste. Because of the perishability of milk and the nature of milk production and handling procedures, the development of off-flavors/odors is not uncommon. To prevent flavor/odor defects in milk, proper milk handling procedures from the farm to the consumer are essential. These defects of milk smell may be classified according to; absorbed/transmitted, bacterial/microbial and chemical/enzymatic processes.6,[10,11,12,21]

Contaminated raw milk may act as a source of many harmful bacteria leading to various diseases, such as undulant fever, Salmonellosis, Dysentery and Tuberculosis. Raw milk with bacteria count below a specified limit is known as "certified" milk and is considered healthy. [2]

The shelf life of pasteurized milk can be affected by large number of somatic cells in raw milk. Increased somatic cell numbers are positively correlated with concentration of plasmin, a heat stable protease and of lipoprotein lipase in freshly produced milk. Activities of these enzymes can supplement those of bacterial hydrolases, hence shortening the time to spoilage. The major determinants of quantities of these enzymes in milk supply are the initial cell numbers of psychotropic bacteria, their generation times, abilities to produced specific enzymes, time and temperature at which the milk is stored before processing. [15]

Methylene blue reduction test is based on the fact that the color imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the color to disappear. The methylene blue reduction test has lost much of its popularity because of its low correlation with other bacterial procedures. This is true particularly in

those samples which show extensive multiplication of the psychotropic species. [23]

The methodology employed the enzymatic reduction of methylene blue by a metabolically active organism turning the Methylene Blue colorless. [24]

Aim of the study: Our aim of this study was to determine quality of kid's milk that purchased from different Markets in Erbil City by using Methylene Blue Dye Reduction Test.

II. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 37 samples comprising of kids milk collected at different levels of collection and processed. Accordingly 12 different milk samples from hypermarket, 8 different milk samples from unlicensed hawker (retail market), 11 different samples with additives from hyper market samples and 6 different samples with high price. Samples were collected. All the samples were kept in an icebox and transported to the laboratory under chilled conditions within 2 hours and analyzed using Methylene blue dye reduction test method as described. [27]

Fig.2.1: (A& B) Different milk samples used in experiment

2.2. Methylene Blue Reduction Test:

All glassware and rubber stoppers Sterilized either in an autoclave or in boiling water. One ml of the Methylene Blue Thiocyanate solution added into a test tube then 10 ml of milk poured into test tube and shacked to mix dye with milk in test tube.

Tubes placed in the water bath immediately for incubation. The temperature maintained at 37 ^{o} C . 25, [28,29]

Fig.2.2: Inoculation of milk samples into methylene blue

The initial time noted down and the test tube examined after half an hour then subsequent readings are taken at hourly interval and results are interpreted as follows:

Classification.–The suggested classification is listed.

Class 1. Excellent, not decolorized in 8 hours.

Class 2. Good, decolorized in less than 8 hours but not less than 6 hours.

Class 3. Fair, decolorized in less than 6 hours but not less than 2 hours.

Class 4. Poor, decolorized in less than 2 hours.

III.   RESULTS

3.1. Sample from supermarket:

Different type of milk that purchased from super market and their quality are shown in Table 3.1 and figure 3.1, All milk types shown no change of methylene Blue colour appear on the base of time, that indicate excellent quality of the milk bellow.
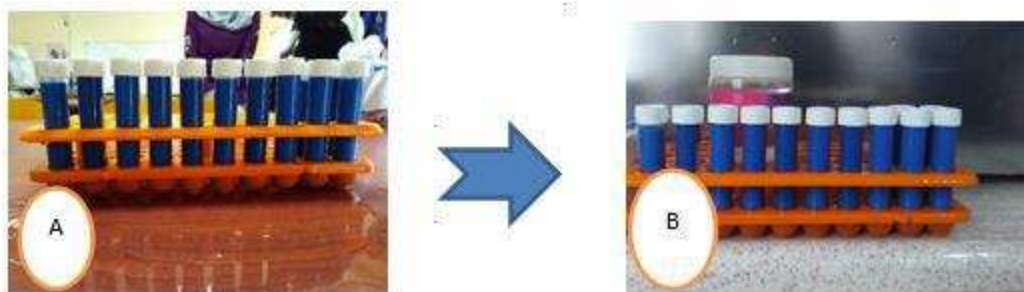

Fig.3.1: Different milk samples purchased from super market (Before and after incubation)

3.2. Samples from Retail Market: After purchased samples from retail market present or absence of bacteria studied and no change of Methylene Blue color appeared by the time table 3.2.and figure 3.2.

Table 3.2. Different milk samples purchased from retail market

No.	Type of Milk sample	Cod	Milk Quantity(ml)	Initial Time (hour)	Final time (hour)
✓	Almarai Milk	Am M	10	9.04 a.m.	-
✓	Yorsan Milk	Yo M	10	9.04 a.m.	-
✓	KDD Milk	K M	10	9.04 a.m.	-
✓	Hamaouda Milk	H M	10	9.04 a.m.	-
✓	Selin Milk	Se M	10	9.04 a.m.	-
✓	Alrabie Milk	Ar M	10	9.04 a.m.	-

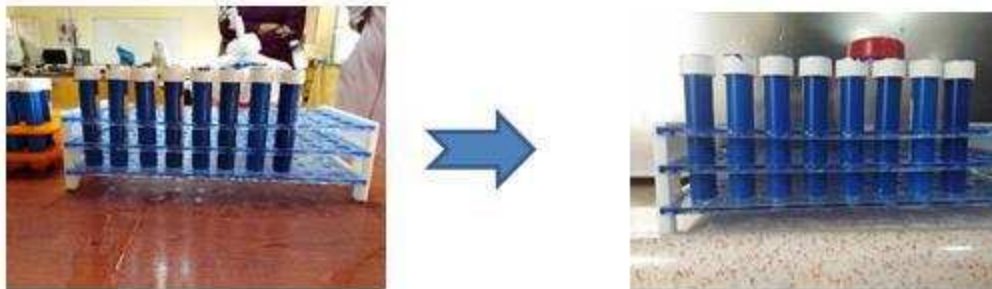| ✓ | Sutas Milk | Su M | 10 | 9.04 a.m. | - |
| ✓ | Sa Milk | Sa M | 10 | 9.04 a.m. | - |

Fig.3.2: Different milk samples purchased from retail market

3.3. Samples from high per market with (High price 500-1000ID)

After purchased different type of milk with high price no change of colour obtained by time as shown in Table 3.3 and figure 3.3.

Table.3.3: Different milk samples from high per market (High price 500 ID)

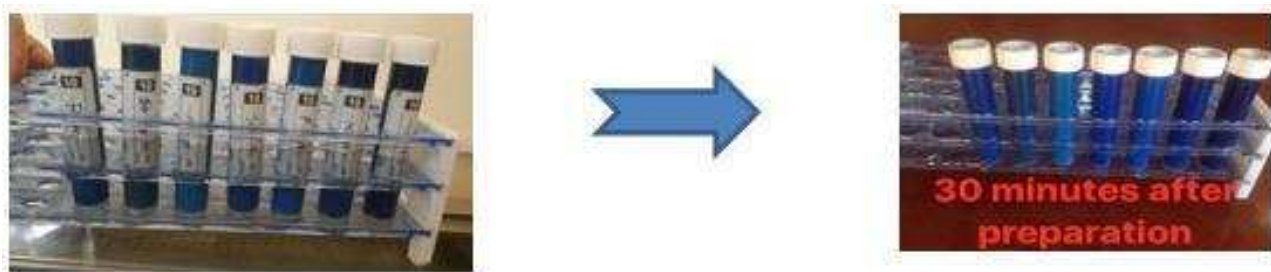| No	Milk	Cod	Milk Quantity(ml)	Initial Time (hour)	Final time (hour)
1.	Shizer milk	Sh M	10	9.04 a.m.	-
2.	Kalleh milk	K M	10	9.04 a.m.	-
3.	Pinar Kido milk	Pk M	10	9.04 a.m.	-
4.	Safio milk	SaM	10	9.04 a.m.	-
5.	Nada milk	N M	10	9.04 a.m.	-
6.	Bouny milk	B M	10	9.04 a.m.	-

Fig.3.3: Different milk samples from high per market (High price 500 ID)

3.4. Sample of milk with different additives:

As shown in table and figure 3.4. After incubation of samples with methylene blue the color of Almarai (Chocolate) changed after 1.5 hours. So tests triplet by purchased samples from different market to confirm the result. That showed no change of color in confirm test.

Table.3.4: Different milk samples with different additives

No	Milk	Cod	Milk Quantity(ml)	Initial time(hour)	finalTime (hour)
1.	Pinar Kido (Strawberry)	Pk S	10	8.40 a.m.	-
2.	Pinar Kido (Chocolate)	Pk Ch	10	8.40 a.m.	-
3.	Kalleh (Banana)	K B	10	8.40 a.m.	-
4.	Kalleh (Straw berry)	KS	10	8.40 a.m.	-

5.	Kalleh (Chocolate)	K Ch	10	8.40 a.m.	-
6.	Almarai nijoom (Strawberry)	Am S	10	8.40 a.m.	
7.	Almarai (Banana)	Am B	10	8.40 a.m.	-
8.	Almarai (Chocolate)	Am Ch	10	8.40 a.m.	10.20a.m.
9.	Alarabie (Banana)	Ar B	10	8.40 a.m.	-
10.	Yorsan (Strawberry)	Yo. S	10	8.40 a.m.	-
11.	Yorsan (Chocolate)	Yo. Ch	10	8.40 a.m.	-

Fig.3.4: Different milk samples with different additives before and after addition of MB.

Sample of Almarai Chocolate from another market

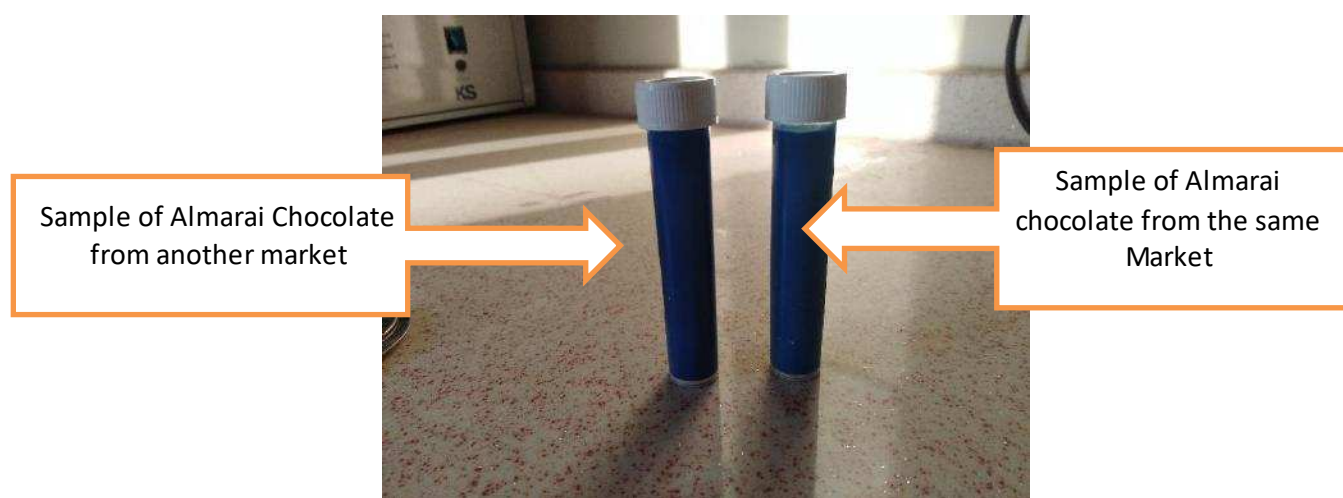Sample of Almarai chocolate from the same Market

Fig.3.5: Repeat the test above to confirm the result by taking sample of milk from different source.

IV.   DISCUSSION

Milk is valuable and consumed on daily basis. As milk contains fat, protein, carbohydrates, minerals, vitamins and other various ingredients dispersed in water, it is considered as a complete diet. at the same time, it is highly vulnerable to bacterial contamination and hence is easily perishable. [30]

Some of these bacteria that grow in milk, during the production of metabolites, may cause an unacceptable sensory alteration, such as off flavor, odor, change in texture or appearance, termed as spoilage. [31,32]

These microorganisms should be termed as specific spoilage organisms for milk, as other microorganisms may also grow in milk but without causing any sensory changes.

The microbial quality of raw milk is important for the production of dairy products and it also influences their shelf life. [33]

Milk of high quality contains a few hundreds of bacteria per milliliter whereas bad milk contains millions of bacteria per milliliter. [34]

The presence of coliform bacteria in milk points out fecal contamination. [35]

Methylene Blue Dye Reduction Test, commonly known as MBRT test is used as a quick method to assess the microbiological quality of raw and pasteurized milk. This test is based on the fact that the blue color of the dye solution added to the milk get decolorized when the oxygen present in the milk get exhausted due to microbial activity. In our study when collect sample of milk that from super market and used Methylene Blue Reduction tests to determine their quality as shown in Table (3.1 -3.2) and figure (3.1-3.2). All milk types shown no change of Methylene Blue color appear on the base of time, that indicate excellent quality of the milk bellow repeated the test to confirm the results and get the same result the sample were collected from supermarket low price (250 ID).

Collected high price kids milk (500-1000ID). After addition of Methylene Blue into the milk samples and detect the quality using time as showed in table 3.3 and figure 3.3. That the color of samples never changed by the time this indicate that the quality of the samples that collected from super market was in excellent quality. In both tests can see that the milk samples was posturized and stored in a good conditions such as temperature and external damage of packet or bottle that the milk present in it.

When studied the quality of milk by using the same method Methylene blue reduction test for the kid milk with additive materials such as addition of fruit or flavors into the milk we found that one of the samples as showed in table 3.4. and figure 3.4 the color of one of the milk with chocolate flavor changed after 2 hours that indicate present of bacteria and reduced the coloure by breakdown the methylene blue colure and the chocolate color appeared to confirm this test we repeated the test by getting the same sample from same market and another market . found that color never changed because of change the color in the first test is one of the factors that cause spoilage of milk such ad damage of milk bottle or present a pore in the milk sample that help inter of bacteria and grow in the milk sample.

When done experiments of the types of milk on the market were changing the color to white means that were contamination. Emphasis of contamination repeats the test for same type of the milk (chocolate) in the same market it does not change the color. It meaning that were not contamination may the first time contamination. When transfer from the factory to the market or services drop of the milk cause vent packed of the milk, may not well storage.

From the study that done by on raw, pasteurized UHT milk samples collected from different locations in Bangladesh assay it can be concluded that the microbial were not satisfactory as indicated by their high bacterial loads and presence of coliform bacteria. However after pasteurization and UHT treatment they were found to be safe for the consumers. Even after this, proper refrigeration temperature should be maintained particularly in case of detection of the quality of the milk using MBR test. [36]

Collected 240 raw milk samples and 72 pasteurized milk samples from different places of Madurai District for a period of six months and were analyzed for microbial quality. Among the raw milk samples only 19.1% of samples were good quality and 28.3% are poor quality. In the pasteurized milk samples 81.9% of samples were excellent for human consumption.[11]

V. CONCLUSION

- From this research work, it is clearly seen that all type of kids milk were sterile and no bacteria detect in milk.
- Its save to consume all type of kids milk that examined in the research with care to storage condition that include storage temperature.
- Present or absence of pore on the surface of milk can or containers.

VI. RECOMMENDATION

1- Using plate count and molecular studies to confirm the test.
2- Study on Detection of adulteration in milk Most of the chemicals used as adulterants are poisonous and cause health hazards.
3- Our recommendations for consumers obtain milk that keep in refrigerator because combination of preservative coupled with subjection to pasteurization and refrigeration could help in extending the shelf-life.
4- Checking expiration date.
5- Checking storage temperature.
6- Checking tetra pack products have vent or concavity.
7- Sure the milk product protective to sun light.
8- Milk products put into sterile tetra pack shelf-safe carton when opened sure it storage in clean place.

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