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Qualitative Analysis of Various Adulterants and Microbes in Milk Samples Collected From Different Areas of Mumbai

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ABSTRACT: The study was carried out keeping the view of recently emerging concern of adulteration of natural milk with various illegal substances to increase its marketability. Milk forms a significant part of diet for all age groups and important source of nutrient required for growth in infants and children for maintenance of health. But the pure nutritious milk is not reached to the people because of milk adulteration. Since milk provides essential nutrients for excellent growth of many microbes, contamination of milk is very frequent which causes various diseases to human health. Qualitative analysis was carried out on 10 milk samples; standardmilk adulteration methods followed by FSSAI (Food Safety and Standard Authority of India)2012 Manual .Hence checking for adulterants and microbial load present in milk was the main objective of this study. Chemical analysis was carried out for detection of adulterants and microbial load was determined by microbiological tests such as SPC, Coliforms count followed by quality check including Methylene blue and Resazurin dye Reductase test (MBRT and RRT). Formalin, Ammonium sulphate, Glucose, Detergents and Benzoic acid were detected as adulterants in milk samples. Water was also detected majorly in some of the milk sample. The microbiological analysis was significantly high in case of loose milk samples than brand milk. Varying results were observed for dye reduction tests. This concludes that the milk analysis and testing should meet all FSSAI standards to be safe for consumption and pasteurization is necessary to avoid bacterial contamination of milk.

KEYWORDS: Adulterants, SPC, MBRT, RRT, FSSAI, Pasteurization

I. INTRODUCTION

Milk is the nature's most nearly perfect food supplying wide range of nutrients and has a very high nutrient density in relation to calorie content of the food. The human body is in need of milk throughout the life as it is useful for all ages and groups. It has all the substances needed by the organisms in its easiest assimilable form. Hence milk is a perfect food, readily digested and absorbed. It is chiefly a valuable source of good quality protein, fat, carbohydrates, vitamins and minerals. Milk is high in nutrition and majority of Indian population rely on milk for their protein supplement. When it leaves the udder of the animal, all the interplay's of adulteration begin. The term adulteration in general may be defined as the addition of some of the legally prohibited substances into a more valuable genuine product. According to PFA act 1954 "adulterant" means any material, which is or could be employed for the purpose of adulteration.Quality of milk is deteriorated due to its adulteration in different marketing channels.Adulteration of milk and there were reports of adulteration in dairy products also in many parts of India. (Ramya P, Feb 2015). The nature of adulterants generally encountered in milk and milk products are water, removal of fat, addition of skim milk powder, reconstituted milk, thickening agents such as starch, flour, glucose, urea, salt, chlorine. Preservatives such as neutralizers which usually consists of sodium bicarbonate, sodium carbonate, sodium hydroxide and calcium hydroxide. (MK, 2014). Fertilizers



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or additives such as Urea, Ammonium sulphate, glucose, Water and Detergents are also added to the milk. To increase the shelf life of milk preservatives such as Benzoic acid, Salicylic acid, Formalinand hydrogen peroxide are added. As a result of these malpractices, the ultimate victim is a consumer, who innocently takes adulterated milk. These all adulterants and Preservatives in excess amounts have very serious health effects on human body causing various types of diseases and other complications. Being an essential food for human beings, milk also acts as a good medium for the growth of many microorganisms.

Microbial contamination of raw milk can occur from various sources like air, milking equipment, feed, soil, grass and feces. In appropriate conditions milk can act as a carrier of disease from milking animals to human via microorganisms. (Muhammad Naseer Abbas, July-Aug 2013). Despite food legislation, adulteration remains uncontrolled, furthermore legal steps laid down in the PFA Act are extremely difficult to maintain due to inadequate and untrained man power and laboratory facilities. Such is the state in the country where we are one of the largest nations of milk producers. In the year 2010-2011, India was ranked among the top 5 countries in the world producing 121.8 million tons of milk. (MK, 2014). The aims and objectives of the study was to carry out Qualitative analysis of various adulterants and determination of microbiological quality of milk of 5 loose and 5 brand milk samples collected from different areas of Mumbai.

II. RELATED WORK

Adulteration of milk is done right after milking and certain factors like animal mishandling; feeding, unhygienic milking, transportation and poor storage conditions are responsible for deterioration of milk. The National Survey on milk adulteration 2011, survey was conducted to check contaminants in milk. Studies found that due to lack of hygiene and sanitation in milk handling and packaging, detergents were not washed properly and traces were found in milk. 70% of milk does not confirm standards set of milk. Problems were more pronounced in milk sold loose as compared to packaged milk. FSSAI (Food Safety Standards Authority of India) has asked all its state and union territories enforcement divisions to strengthen checks on milk producers to ensure they are complying with Food Safety and Standards Act. In another case study where the Food and Drug administration department in Thane registered a case against six well-known companies, the milk sample contained added sugar which may affect health of any diabetic patient when consumed such type of milk. Sometimes milk samples are prepared with added adulterants during the production and processing or added intentionally according to one's own choice to generate money Sometimes people think that buying packaged, branded milk was a safeguard against adulteration. But in June 2015 in Mulund, a milkman was arrested for selling adulterated milk in packets after addition of 10 parts of impure water into 3 parts of milk. In turn he would convert 30 liters of milk into 100 liters of milk. Study carried out in year 2010 found out that 68.4% samples were adulterated across the country. Only Goa and Pondicherry state confirmed the required standards for all samples, whereas states like West Bengal, Bihar, Chhattisgarh, Jharkhand, Orissa, Mizoram not a single sample met the norms. Other states fared a shade better. Samples were collected randomly and analyzed from 33 states totaling a sample size of 1,791. Just 31.5% of samples tested (565) confirmed to FSSAI standards while the rest 1,226 (68.4%) failed the test. This study conducted by FSSAI across 33 states found that milk was adulterated with detergent, fat and even urea and water also.

The safety of milk is an important attribute for consumers of milk and dairy products. The presence of food borne pathogens in milk is due to direct contact with contaminated sources in the dairy farm environment and due to excretion from the udder of an infected animal. Entry of food borne pathogens via contaminated raw milk into dairy food processing plants can lead to persistence of these pathogens in bio films, and subsequent contamination of processed milk products and exposure of consumers to pathogenic bacteria. This is especially true in developing countries where production of milk and various milk products takes place under unsanitary conditions and poor production practices. A major factor determining milk quality is its microbial load. It indicates the hygiene practiced during milking, like cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal. An Article was published in 2015 based on thestudy to determine the prevalence and antimicrobial resistance of *Staphylococcus aureus* isolated from raw milk (cow and sheep) and dairy products (traditional cheese in Mazandaran Province, Iran). A total of 2650 samples, including 1930 raw milk and 720 dairy products were purchased from retail stores. Out of 2650 samples, *S. aureus* was detected in 328 samples (12.4%)



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in which 53 (16.2%) were positive for methicillin-resistant *S. aureus*. The *S. aureus* isolates showed resistance to tetracycline (56.1%), followed by penicillin G (47.3%), oxacillin (16.2%), lincomycin (11.9%), clindamycin (11.3%), erythromycin (7.9%), streptomycin (5.8%), cefoxitin (5.5%), kanamycin (4%), chloramphenicol (3.7%), and gentamicin (2.1%). A high frequency of *blaZ* (46%) and *tetM* (34.8%) resistance genes was found in *S. aureus* isolates. The findings of this study revealed consumption of raw milk and dairy products as a potential risk of foodborne infection in this region. Another article was published in 2014, Milk Adulteration in Hyderabad, India - A Comparative Study on the Levels of Different Adulterants Present in Milk. The study was carried outkeeping in view the recently emerging concern of adulteration of natural milk with various illegal substances to increase its marketability. This study explains in detail the hygienic status of milk supplied to various cafes, small hotels and other public and educational institutions. Qualitative analyses were carried out on 50 milk samples; and a standard milk adulteration kit manufactured by Himedia laboratories, Mumbai, India was used.

Presented are the significant observations of the study: Sucrose and skim milk powder were present in 22% and 80% of the milk samples, respectively. Urea, neutralizers, and salt were present in 60%, 26% and 82% of the milk samples, respectively. Formalin, detergents, and hydrogen peroxide were present in 32%, 44%, and 32% of the milk samples obtained. Consequently, this qualitative analysis proved that the milk procured did not confirm to the legal standards and was adulterated with toxic chemicals, which are injurious to health.

III. MATERIALS AND METHODS

I. CHEMICAL ANALYSIS:-

1. Test For Detection Of Neutralizers:-

Rosolic acid test (Soda Test)-

•Take 5 ml of milk in a test tube and add 5 ml alcohol followed by 2-3 drops of rosolic acid. If the color of milk changes to pinkish red, it is inferred that the milk is adulterated with sodium carbonate /sodium bicarbonate and so unfit for human consumption.

2. Test For Detection Of Hydrogen Peroxide:-

• Take 5 ml milk in a test tube. Add 3 drops of paraphenylene diamine and shake well. Change in color of the milk to blue confirms that the milk is adulterated with hydrogen peroxide.

• To 10 ml of milk sample in a test tube add 10-15 drops of Vanadium Pent oxide reagent and mix. The development of pink or red color indicates presence of hydrogen peroxide.

3. Test For Detection Of Formalin:-

Formalin (40%) although poisonous, can preserve milk for a long time.

•Take 10 ml of milk in a test tube. Add 5 ml conc. sulphuric acid through the sides of the test tube without shaking. If a violet or blue ring appears at the intersection of the two layers, it shows the presence of formalin. Note violet coloration usually does not appear when relatively large quantities of formaldehyde are present.

4. Test For Detection Of Cane Sugar:-

Generally cane sugar is mixed in milk to increase the percentage solids content of milk i.e., to increase the lactometer reading of milk, that was already diluted with water.

• Take 10 ml of milk in a test tube. Add 5 ml of hydrochloric acid along with 0.1 g of resorcinol. Shake the test tube well and place it in a boiling water bath for 5 min. Appearance of red color indicates the presence of added cane sugar in milk.

5. Test For Detection Of Starch:-

Addition of starch increases the SNF content of milk. Wheat flour, arrowroot, rice flour, etc., can also be added for increasing the SNF content.

• Take 3 ml milk in a test tube and boil it thoroughly. Cool the milk to room temperature. Add 2 to3 drops of 1% iodine solution. Change of color to blue indicates that the milk is adulterated with starch.



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6. Test For Detection Of Glucose:-

Poor quality glucose is sometimes added to milk to increase the lactometer reading.

• Take 3 ml of milk in a test tube. Add 3 ml Barford's reagent and mix it thoroughly. Keep the test tube in a boiling water bath for 3 min and then cool it for 2 min by immersing it in tap water without disturbance. Add 1 ml of phosphomolybdic acid and shake. If blue color is visible, then glucose is present in the milk sample.

7. Test For Detection Of Urea:-

Urea is generally added in the preparation of synthetic milk to raise the SNF value.

• 5 ml of milk is mixed well with 5 ml paradimethyl amino benzaldehyde reagent. If the solution turns distinct yellow in color, then the given sample of milk contains urea. Control, normal milk may show a faint yellow color due to presence of natural urea.

8. Test For Detection Of Ammonium Sulphate:-

The presence of sulphate in milk increases the lactometer reading.

• Take 5 ml of milk add 2.5 ml of 2% sodium hydroxide, 2.5 ml of 2% sodium hypochlorite and 2.5ml of 5% phenol solution then heating for 20 seconds in boiling water bath. If bluish color turns to deep blue it indicates the presence of ammonium sulphate, however in case it turns to pink it shows that the sample is free from Ammonium sulphate.

9. Test For Detection Of Salt:-

Addition of salt in milk is mainly resorted to with the aim of increasing the corrected lactometer reading.

• 5 ml of silver nitrate reagent is taken in a test tube. Add 2-3 drops of potassium dichromate reagent. Add 1 ml of milk in the above test tube and mix thoroughly. If the contents of the test tube turn yellow in color, then milk contains salt. If it turns to chocolate or reddish brown in color, the milk sample is free from salt.

10. Detection of Detergents:-

• Take 5 ml of milk in a test tube and add 1-2 drops of bromocresol purple solution. Mix well. Appearance of violet color indicates the presence of detergent in milk. Unadulterated milk samples will show a very faint violet coloration.

11. Test For Water :-

Lactometer reading detects adulteration of milk with water. Take raw milk in a long stemmed wide mouth bottle or a measuring cylinder. Place the lactometer in it taking care to see that the lactometer does not touch the sides of the bottle or the measuring cylinder. Note down the reading at the surface of milk sample taken.

12. Detection of Benzoic And Salicylic Acid:-

• Take 5 ml of milk in a test tube. Add 3-4 drops of concentrated sulphuric acid. Add 0.5% ferric chloride solution drop by drop and mix well. Development of buff color indicates presence of benzoic acid and violet color indicates presence of salicylic acid.

II. MICROBIAL ANLAYSIS

1. STANDARD PLATE COUNT (SPC):-

Standard Plate Count is a method used for determining the total number of viable bacteria in milk samples. Milk is serially diluted 10-fold using saline to reduce the load of microbial growth and to obtain well isolated colonies followed by spread plate technique. After making dilutions, plate out 0.1ml on to 10^{-4} , 10^{-5} and 10^{-6} on Nutrient Agar which supports the growth of all organisms. Incubation is done at ambient temperature for 24-48hr. Observe and counts the colonies from all plates and interprets by comparing to standard result table.



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INTERPRETATION TABLE:-

Grade
Very Good
Good
Fair
Poor

2. COLIFORM COUNT:-

It is important in quality control of milk as it is indicative of possible fecal contamination and presence of pathogens. For pasteurized milk it indicates post pasteurization contamination. Absence of coliforms in 1:100 dilution of raw milk or 1:10 dilution of pasteurized milk is considered satisfactory. Dilutions are made and pated out 0.1ml on 10^{-1} , 10^{-2} plates. Coliforms count is performed using Violet Red Bile Agar incubated at 37°C for 24 hr. Violet colonies are indicative of coliforms.

3. METHYLENE BLUE REDUCTASE TEST:-

The organism present in the milk reduces Methylene blue to a colorless compound. The time taken to reduce the Methylene blue by a given sample of milk indicates the quality of milk. The sample which shows decolourisation of blue dye in 30 mins at 37° C is not acceptable as per standards. Incubate all tubes at 37° C. Note the time for decolourisation in the **TEST** tube.

INTERPRETATION TABLE:-

Time for Decolourisation	Interpretation
8 hrs. and above	Excellent
6-8 hrs.	Good
2-6 hrs.	Fair
Less than 2 hrs.	Poor

4. RESAZURIN REDUCTASE TEST:-

This is a redox dye which changes color as the pH of the milk changes due to growth of organisms. At the same time the dye is also reduced in 2 stages. In the first stage an irreversible change from the blue resorufin and then finally to the colorless dihydroresorufin takes place. The test is intended as a platform test for detecting milk of poor quality.Incubate all tubes at 37°C for 30mins for color change.

INTERPRETATION TABLE:-

Color	Quality
Blue/Lilac	Good
Mauve/Pink	Fair
Pink/Colorless	Poor

IV. RESULTS AND DISCUSSION



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The loose milk samples were collected from Goregoan, Bhayander, Kandivali and Church gate and brand milk samples were collected from different dairies.

I. CHEMICAL ANALYSIS

SR.NO	ADULTERANTS	L-1	L-2	L-3	L-4	L-5	B-1	B-2	B-3	B-4	B-5
	PRESERVATIVES										
1)	Formaldehyde	-	+	-	-	_	_	-	-	1	I
2)	Benzoic Acid	+	+	+	+	+	+	+	+	+	+
3)	Salicylic Acid	_	_	_	_	_	_	_	_	_	_
4)	Hydrogen Peroxide	-	-	-	-	-	-	-	-	_	-
	NEUTRALIZERS										
1)	Sodium Carbonate/ Bicarbonate	_	_	_	_	_	_	_	_	_	_
	THICKENING AGENTS-										
1)	Starch	-	-	-	_	_	_	-	-	-	-
2)	Cane Sugar	-	-	-	-	_	_	+	_	_	-
	FERTILIZERS/ ADDITIVES-										
1)	Urea	-	-	-	-	_	_	_	_	_	-
2)	Ammonium Sulphate	+	-	-	I	+	_	-	+	+	+
3)	Glucose	_	+	+	+	_	+	+	_	_	+
4)	Salts	_	-	-	_	_	_	_	_	_	_
5)	Water	30	29	30	28	20	30	30	32	30	31
6)	Detergents	-	_	_	_	_	_	_	_	_	+

KEY: - '+' –POSITIVE, '–' - NEGATIVE



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Fig 1. LOOSE MILK SAMPLE -2 (a) Formalin is present as the milk shows violet coloration at the bottom of the test tube number-3 and other adulterants like starch, cane sugar, hydrogen peroxide and sodium carbonate/bicarbonate are absent. (b) Glucose is present as the milk changes to blue color in test tube number- 5 and other adulterants like detergents, salt ammonium sulphate and ureaare absent. (c) Benzoic acid is present as milk shows buff color and salicyclic acid is absent. (d) Lactometer reading is 29 indicating milk is not adulterated by water.

II. MICROBIOLOGICAL ANALYSIS

Sr.No	Samples	SPC	Interpretation
1)	L-1	2.0X10 ⁶	Fair
2)	L-2	2.6X10 ⁸	Poor
3)	L-3	5.9X10 ⁷	Poor
4)	L-4	2.9X10 ⁹	Poor
5)	L-5	9.2X10 ⁷	Poor
6)	B-1	3.9X10 ⁷	Fair
7)	B-2	1.0X10 ⁹	Poor
8)	B-3	2.7X10 ⁸	Poor
9)	B-4	9.2X10 ⁷	Poor
10)	B-5	1.1X10 ⁸	Poor

1) STANDARD PLATE COUNT-

2) COLIFORM COUNT-

SR.NO	SAMPLES	COLIFORM COUNT
1)	L-1	$2.7 X 10^{5}$
2)	L-2	TNTC
3)	L-3	$1.08 X 10^{6}$
4)	L-4	TNTC
5)	L-5	9.8X10 ⁴
6)	B-1	$2.9X10^{5}$
7)	B-2	TNTC



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8)	В-3	4.6X10 ⁴
9)	B-4	1.8X10 ⁵
10)	B-5	4.8×10^{5}

KEY:-TNTC- TOO NUMERABLE TO COUNT



Fig 2. BRAND MILK SAMPLE-5 (a) According to Interpretation table, Standard Plate Count was high than 5x10⁷ indicating poor quality of milk. (b) According to standard table, absence of coliforms in 1:100 dilution of raw milk or 1:10 dilution of pasteurized milk is considered satisfactory.But the coliforms count on above table and in the image is very high in all milk samples which indicate high risk of fecal contamination and thus proper hygienic practice needs to be maintained. Therefore results here are unsatisfactory.

3) METHYLENE BLUE REDUCTASE TEST-

Sr.No	Samples	Test	C-1	C-2	Interpretation
1)	L-1	С	В	С	Good
2)	L-2	С	В	С	Fair
3)	L-3	С	В	С	Fair
4)	L-4	С	В	С	Good
5)	L-5	В	В	С	Excellent
6)	B-1	С	В	С	Good
7)	B-2	В	В	С	Excellent
8)	B-3	В	В	С	Excellent
9)	B-4	В	В	С	Excellent
10)	B-5	С	В	С	Fair

KEY: - C – COLORLESS, B- BLUE



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4) RESAZURIN REDUCTASE TEST-

Sr.No	Samples	Test	C-1	C-2	Interpretation
1)	L-1	Pi	Pi	С	Fair
2)	L-2	Pi	С	С	Fair
3)	L-3	Pi	Pi	C	Fair
4)	L-4	С	Pi	С	Poor
5)	L-5	Li	Pi	С	Good
6)	B-1	Li	Pi	С	Good
7)	B-2	Pi	Pi	С	Fair
8)	B-3	Li	Pi	С	Good
9)	B-4	Li	Pi	С	Good
10)	B-5	Pi	C	С	Fair

KEY: - Li-GOOD, Pi-PINK, C-COLORLESS



Fig 3. LOOSE MILK SAMPLE-1 (a) According to Interpretation table, the MBRT result indicates good quality of milk as the time of decolourisation was between 6-8 hours (b) RRT result indicates fair quality as the color of the milk changed to pink.

V. CONCLUSION

Qualitative Analysis was carried out to check various adulterants and their microbiological quality of loose and brand milk collected from different areas of Mumbai. On the basis of data obtained in the present study, conclusion may be drawn that milk quality is not completely as per standards and adulteration in milk is still in practice and has not been checked completely. In a country such as India where milk and milk products play an important role in different foodstuffs, this analysis carried out will bring about more awareness to the general public about the malpractices and negligence during milk production and transportation. In adulterants, **Formaldehyde**was detected in one of the sample whereas**Benzoic Acid** was detected in all samples of milk. They serve us preservatives in the milk for increasing shelf life of milk and preventing it from sour or bitter taste. Natural preservatives such as honey can also be used to reduce chemical preservatives during milk storage. Further quantitative analysis is required to find out the quantity of preservatives causes renal failure, respiratory disease, vomiting, metabolic acidosis etc. **Ammonium sulphate** was detected in three of brand (B-3, B-4 and B-5), loose milk samples (L-1 and L-5). It serves as Additives which is added



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to increase the lactometer reading by maintaining density of milk. On higher amounts it can cause severe irritation and inflammation of respiratory tract.

Glucose was detected in three loose milk (L-2, L-3 and L-4) and brand milk samples (B-1, B-2 and B-5). The poor quality glucose is added to increase carbohydrate content of the milk and thus density is also increased. Due to this the milk can now be adulterated with water and it will not be detected by lactometer test. Detergent was detected in one of the brand milk sample(B-5) which indicates negligence of improper washing of utensils, boilers, storage tanks during production and transportation of milk.Loose milk sample -5 showed significant results as lactometer reading was below 30. This indicates milk is adulterated with water. The source of water also is of great matter. Contaminated water used for adulteration can lead to serious health related infections and diseases. The microbiological nature of loose and brand milk was poor. Hence hygienic conditions need to be maintained right from the collection centre to the consumers. The coliforms count was very high as compared to standard table which indicates high level of risk. The presence of coliforms indicates fecal contamination which can take place easily due to unhygienic maintenance of milk in the cattle. The Dye Reduction Tests also gave intermediate results which were not too good or too bad. Handling of milk from cattle, production stage where it passes through various steel pipes, boilers etc. till storage and transportation has to be maintained properly. Pasteurisation is very much important before consumption. To ensure that good quality milk is reached to the consumers, there should be proper quality control system. This will improve the quality and reputation of milk entities which increases the profits as well as protects health of animals and consumers of milk.

VI. FUTURE PROSPECTS

Milk and milk products need to be analyzed for variety of reasons such as assessment of milk quality, determination of nutritive value, detection of adulteration and in research and development. Government bodies regulate the permitted levels of certain preservatives and contaminant compounds; much of this advancement has been driven by increased sensitivity and specificity of determination e.g. using analytical instruments. But sometimes it becomes difficult and impossible to accurately analyses one component in the presence of others using classical method of analysis. Due to lack of specificity and sensitivity of classical method of analysis it may lead to inaccurate, unreliable results, erroneous and false results. Therefore in order to achieve the reliability of results, nowadays instrumental analytical techniques have become mandatory in quality control, safety and meeting the regulatory norms. Selecting appropriate instrumental technique is also very important because criteria such as Precision, Reproducibility, Accuracy, Speed, Sensitivity, Specificity needs to be taken into consideration. Analytical Balance, Electric Ovens, Muffle Furnace, pH Meter, Infra-Red Moisture Balance, Laminar Air Flow Chamber, Freeze Dryer (Lyophilizer), Centrifuge and Cream Separator are the common laboratory instruments used during analysis of milk products in any laboratory. Some other used instruments that are frequently used in quality control lab are as follows: Milko-tester, Infra-red Milk analyzer (Milk scan), FTIR-Milko-scan, Lacto star Automatic Milk Analyzer, Pro-Milk MK II, Polari meter, Butyro Refractometer, Automated Kjeldahl for Protein Estimation (Kjeltec). Besides common equipment's certain Sophisticated instruments are also available that help in detection of adulterants and quantification of bio-actives. These sophisticated instruments are follows: Thin layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPLTC), Gas Liquid Chromatography (GLC), High Performance Liquid Chromatography (HPLC), Mass Spectrometry, LC-MS, GC-MS, Texture profile analyzer, Spectrophotometer, Flame Photometer, Atomic Absorption Spectrophotometer, Rancimat. By using such instruments not only adulterants can be detected but also the protein value, fat value and other nutritive information can be determined.

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