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Nutritional, Biochemical, Microbial assessment & in vitro antimicrobial activity of Milk at various stages of processing

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Abstract
The milk intended for human consumption must meet the terms of safety and human security. The purpose of this dissertation was to evaluate the raw milk quality with pasteurized milk. A variety of diagnostic tests are routinely used to evaluate milk quality on dairy farms. Tests such as bulk milk bacterial counts, bulk tank somatic cell count and tests for adulterants such as water, sediment or antibiotics are routinely used. This study reviews practical applications and supporting research of tests that are commonly used to investigate and solve milk quality and microbial load on dairy practices.

Keywords: somatic cell, adulterants, pasteurization

Introduction
Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for infant mammals (including humans who breastfeed) before they are able to digest other types of food.
Throughout the world, more than six billion people consume milk and milk products. Over 750 million people live in dairy farming households. In almost all mammals, milk is fed to infants through breastfeeding, either directly or by expressing the milk to be stored and consumed later.
Colostrum the early milk from mammals contains antibodies that provide protection to the new born baby as well as nutrients and growth factors. The makeup of the *Colostrum* and the period of secretion vary from species to species.

Recommendations of WHO
For humans, the World Health Organization recommends exclusive breastfeeding for six months and breastfeeding in addition to other food for at least two years. In some cultures it is common to breastfeed children for three to five years, and the period may be longer.

Properties of milk
Milk is an emulsion or a colloid of butterfat globules within a water-based fluid that contains dissolved carbohydrates and protein aggregates with minerals. Because it is produced as a food source for the young ones all of its contents provide benefits for growth. The principal requirements are energy (lipids, lactose, and protein), biosynthesis of non-essential amino acids supplied by proteins (essential amino acids and amino groups), essential fatty acids, vitamins and inorganic elements, and water are its notable contents.

pH
The pH of milk ranges from 6.4 to 6.8 and it changes over time. Milk from other bovines and non-ovine mammals varies in composition, but has a similar pH.

Lipids
Initially milk fat is secreted in the form of a fat globule surrounded by a membrane. Each fat globule is composed almost entirely of triacylglycerols and is surrounded by a membrane consisting of complex lipids such as phospholipids, along with proteins. These act as emulsifiers which keep the individual globules from coalescing and protect the contents of these globules from various enzymes in the fluid portion of the milk. Although 97–98% of lipids are triacylglycrols, small amounts of di- and monoacylglycerols, free cholesterol and cholesterol esters, free fatty acids, and phospholipids are also present. Unlike protein and carbohydrates, fat composition in milk varies widely in the composition due to genetic, lactational, and nutritional factor difference between different species.

Proteins
Normal bovine milk contains 30–35 grams of protein per liter of which about 80% is arranged in casein micelles. Total proteins in milk represent 3.2% of its composition

Caseins
The largest structures in the fluid portion of the milk are "casein micelles": aggregates of several thousand protein molecules with superficial resemblance to a surfactant micelle, bonded with the help of nanometer-scale particles of calcium phosphate. Each casein micelle is roughly spherical and about a tenth of a micrometer across. There are four different types of casein proteins: αs1-, αs2-, β-, and κ-caseins. Collectively, they make up around 76–86% of the protein in milk, by weight. Most of the casein proteins are bound into the micelles. There are several competing theories regarding the precise structure of the micelles, but they share one important feature: the outermost layer consists of strands of one type of

protein, k-casein, reaching out from the body of the micelle into the surrounding fluid. These kappa-casein molecules all have a negative electrical charge and therefore repel each other, keeping the micelles separated under normal conditions and in a stable colloidal suspension in the water-based surrounding fluid.

Salts, Minerals, and Vitamins

Minerals or milk salts are traditional names for a variety of cations and anions within bovine milk. Calcium, phosphate, magnesium, sodium, potassium, citrate, and chlorine are all included as minerals and they typically occur at concentration of 5–40 mM. The milk salts strongly interact with casein, most notably calcium phosphate. It is present in excess and often, much greater excess of solubility of solid calcium phosphate. In addition to calcium, milk is a good source of many other vitamins. Vitamins A, B6, B12, C, D, K, E, thiamine, niacin, biotin, riboflavin, folates, and pantothenic acid are all present in milk.

Carbohydrates and Miscellaneous contents

Milk contains several different carbohydrates including lactose, glucose, galactose, and other oligosaccharides. Lactose which is a disaccharide composed of two simple sugars, glucose and galactose gives milk its sweet taste and contributes approximately 40% of whole cow's milk's calories. Bovine milk averages 4.8% anhydrous lactose, which amounts to about 50% of the total solids of skimmed milk. Levels of lactose depends on the type of milk as other carbohydrates can be present at higher concentrations that lactose in milks. Other components found in raw cow's milk are living white blood cells, mammary gland cells, various bacteria, and a large number of active enzymes

Calcium phosphate, a backbone for Micelle

For many years the most accepted theory of the structure of a micelle was that it was composed of spherical casein aggregates, called submicelles that were held together by calcium phosphate linkages. However, there are two recent models of the casein micelle that refute the distinct micellular structures within the micelle.

The first theory attributed to de Kruif and Holt, proposes that nanoclusters of calcium phosphate and the phosphopeptide fraction of beta-casein are the centerpiece to micellular structure. Specifically in this view, unstructured proteins organize around the calcium phosphate giving rise to their structure and thus no specific structure is formed.

Appearance

Both the fat globules and the smaller casein micelles, which are just large enough to deflect light, contribute to the opaque white color of milk. The fat globules contain some yellow-orange carotene, enough in some breeds (such as Guernsey and Jersey cattle) to impart a golden or "creamy" hue to a glass of milk. The riboflavin in the whey portion of milk has a greenish color, which sometimes can be discerned in skimmed milk or whey products. Fat-free skimmed milk has only the casein micelles to scatter light, and they tend to scatter shorter-wavelength blue light more than they do red, giving skimmed milk a bluish tint.

Qualities of processed milk

Processed cow's milk was formulated to contain differing amounts of fat during the 1950s. One cup (250 mL) of 2%-fat cow's milk contains 285 mg of calcium, which represents 22% to 29% of the daily recommended intake (DRI) of calcium for an adult.

The amount of calcium from milk that is absorbed by the human body is disputed. Calcium from dairy products has a greater bioavailability than calcium from certain vegetables, such as spinach, that contain high levels of calcium-chelating agents, but a similar or lesser bioavailability than calcium from low-oxalate vegetables such as kale, broccoli, or other vegetables in the *Brassica*genus.

Milk as a calcium source has been questioned in media, but scientific research is lacking to support the hypothesis of acidosis induced by milk. The hypothesis in question being that acidosis would lead to leeching of calcium storages in bones to neutralize pH levels (also known as acid-ash hypothesis). Research has found no link between metabolic acidosis and consumption of milk.

Recommended Consumption

Standing with the guidance of WHO the U.S. federal government document *Dietary Guidelines for Americans, 2010* recommends consumption of three glasses of fat-free or low-fat milk for adults and children 9 and older (less for younger children) per day. This recommendation is disputed by some health researchers who call for more study of the issue, given that there are other sources for calcium and vitamin D. The researchers also claim that the recommendations have been unduly influenced by the American dairy industry, and that whole milk may be better for health due to its increased ability to satiate hunger.

Additives and Flavoring

In areas where the cattle (and often the people) live indoors, commercially sold milk commonly has vitamin D added to it to make up for lack of exposure to UVB radiation.

Reduced-fat milks often have added vitamin A palmitate to compensate for the loss of the vitamin during fat removal; in the United States this results in reduced fat milks having higher vitamin A content than whole milk.

Milk often has flavoring added to it for better taste or as a means of improving sales. Chocolate milk has been sold for many years and has been followed more recently by strawberry milk and others. Some nutritionists have criticized flavored milk for adding sugar, usually in the form of high-fructose corn syrup, to the diets of children who are already commonly obese in the US.

Antimicrobial properties of milk

Lactoferrin

Lactoferrin is an iron-binding protein that is found in the milk, saliva, and other body fluids of mammals. Purified lactoferrin has been shown in research studies to have some antibacterial activity against Escherichia coli O157:H7, Listeria monocytogenes, and other foodborne pathogens and spoilage organisms.

Purified lactoferrin (>95%) is produced on a commercial scale from skim milk and cheese whey. Although the natural

lactoferrin content of milk is low, the availability of large quantities of milk and whey provide a good source of materials for lactoferrin production. The purification technique uses a high heat pasteurization process (194-212°F (90-100°C) for 5 to 10 min) to inactivate bacteria and viruses that may be present in raw milk. Consequently, the pasteurization conditions used for beverage milk do not destroy the activity of lactoferrin.

Purified lactoferrin is used commercially in infant formula, milk, yogurt, and nutritional supplements. The typical concentration of lactoferrin naturally present in beverage milk is 0.1 g/kg (Walstra *et al.*, 1999). There are currently no reports available in the scientific literature that has evaluated the effectiveness of the natural levels of lactoferrin in milk to prevent against illness from pathogens that may be present in the same milk.

Lactoperoxidase

Lactoperoxidase is one of most heat stable enzymes found in milk. Lactoperoxidase has antibacterial activity when it is combined with hydrogen peroxide and thiocyanate. The lactoperoxidase system has been used to reduce spoilage and extend the shelf-life of raw milk in countries where refrigeration may be unavailable (e.g., India). The lactoperoxidase system has been shown to be effective in reducing the growth of Listeria monocytogenes in raw milk at refrigerator temperatures.

It has been suggested that the presence of lactoperoxidase in raw milk inhibits the disease causing microorganisms (pathogens) present in milk. However, as hydrogen peroxide and thiocyanate must be added to milk in order to activate the system to achieve antibacterial benefits, (since the latter compounds are not naturally present in raw milk), it is unlikely that the lactoperoxidase system contributes significantly to control of pathogens in fresh raw milk.

Materials & Methods

Sources of milk samples

Raw milk sample and the packed milk of Tulya Dairy Farms Pvt Ltd, Naranamangalam, Perambalur were procured and subjected to various analyses.

Sampling of Milk

Several precautions were taken during sampling of milk. The samples were collected in sterile glass stoppered bottles and care was taken not to wet the stopper or the neck of the bottle. The stopper was removed from the bottle until necessary and replaced immediately after the sample was obtained. The bottle was not filled more than three-quarters so that milk might be shaken before examination. The sample must be the representative one of the milk examined. When samples from bulk milk were taken the contents of the milk tank was thoroughly mixed with a sterile plunger and a quantity proportional to the amount of milk in the tank was removed with a sterile dipper. After collection, the samples were cooled and carried for analysis in an ice-box and brought to the laboratory immediately. Representative milk samples were obtained for evaluating its biochemical and microbiological quality. The milk samples were thoroughly mixed by shaking the bottle 25 times with an excursion of about 1 foot for a time occupying approximately 12 seconds to obtain a uniform sample for plating. For microbiological examination, one portion of the sample was preserved in a clean sterilized container and kept in the refrigerator at 4 ± 100.

Biochemical analysis of milk

Determination of pH

pH of all the test samples were determined by systronics pH meter model LI - 120.

Principle

The pH value, or negative logarithm of hydrogen ion concentration, gives a measure of the true acidity of milk. The relationship between pH and acidity of milk is only approximate.The pH test is mainly used for the detection of abnormal mastitis milk (1ST, 1960).

Determination of specific gravity of milk

The specific gravity of milk was determined according to the method followed by (1ST, 1977).

Principle

The specific gravity of milk is the ratio between the weight of certain volume of milk at the standard temperature of 15.6°C and the weight of the same volume of water at the same temperature. Since temperature influences specific gravity, it should be done at standard temperature.

Procedure

The sample of milk to be treated was thoroughly shaken and brought to a temperature between 10°C to 21.VC. The milk was taken in the cylinder and lactometer was lowered into the milk without touching the walls of the cylinder. The lactometer reading was noted.

Determination of moisture in milk

The Moisture content in milk was carried out as per API-IA (1975).

Procedure

A previously weighed, heated and cooled crucible with 10 grams of milk was weighed accurately. The crucible was heated in an hot air oven at 70-100°C for 4 hours. Then the crucible was transferred into a desiccator, cooled, and then weighed. The crucible was placed in the oven for 30 minutes, cooled in a desiccator and weighed. The above procedure was repeated till the concordant value was obtained.

Determination of total solids

Estimation of total solids was carried out according to the method given in APHA (1975).

Principle

The total solid content of the sample determines the solids present in milk

Procedure

A clean, dry, empty silica crucible was weighed with lid. Five milliliter of the representative milk sample was pipetted out into the crucible and was weighed with the lid. Then the

crucible was placed in a water bath. The base of the crucible was kept horizontal to promote uniform drying and it was protected from direct contact with the metal of the water bath. After 30 minutes the crucible was removed, the bottom was wiped and was transferred to a well ventilated oven maintained at 98 - 100°C. After 3 hours the crucible and the lid was transferred to a desiccator. Then the crucible was allowed to cool for 30 minutes and reweighed (W3). The weighing was repeated until the loss of weight between successive weighing does not exceed 0.5 milligram and the lowest reading was noted.

Estimation of fat

The estimation of fat was carried out according to the method given by 1ST (1977).

Principle

When definite quantities of sulphuric acid and amylalcohol are added to definite volume of the sample, protein will be dissolved and the fat globules will be set free, and remain in liquid state due to heat produced by the acid. On centrifugation, fat being lighter will be separated on the top of the solution.

Procedure

Ten milliliter of Gerber sulphuric acid from automatic measure was taken into the butyrometer. Accurately 10.75milliliter of representative milk sample was pipetted out into the butyrometer without allowing the milk to mix with the acid. This was done by allowing the jet of milk from the pipette to hit the inside wall of the butyrometer, by holding the pipette in a slanting manner and resting the tip on the mouth of the butyrometer. With the help of automatic measure one milliliter of amyl alcohol was added to the butyrometer. The stopper was tightened and the contents were mixed well. Then the butyrometer was centrifuged for five minutes at 1200 rpm. After centrifugation the butyrometer was kept in a water bath at 65°C for five minutes. The reading was taken after adjusting the fat column to be within the scale of butyrometer and recorded.

Estimation of titrable acidity

Estimation of titrable acidity was carried out according to the method described in 1SI (1960).

Principle

The acidity is determined by neutralization of the acid by the known amount of standard alkali solution. Normally 0.1 N alkali neutralizes 0.009 gram of lactic acid.

Procedure

Ten milliliter of milk was taken in a clean conical flask and equal quantity of distilled water was added to it, followed by the addition of few drops of phenolphthalein indicator. It was titrated against 0.1 N Sodium hydroxide solution until a faint pink colour persists. The titrations were repeated to get concordant values.

The alcohol test

The test is quick and simple. It is besed on instability of the proteins when the levels of acid and/or rennet are increased and acted upon by the alcohol. Also increased levels of albumen (colostrum milk) and salt concentrates (mastitis) results in a positive test.

Procedure

The test is done by mixing equal amounts of milk and 68% of ethanol solution in a small bottle or test tube. (68 % Ethanol solution is prepared from 68 mls 96% (absolute) alcohol and 28 mls distilled water). If the tested milk is of good quality, there will be no coagulation, clotting or precipitation, but it is necessary to look for small lumps. The first clotting due to acid development can first be seen at 0.21-0.23% Lactic acid. For routine testing 2 mls milk is mixed with 2 mls 68% alcohol.

Organoleptic Tests

The organoleptic test permits rapid segregation of poor quality milk at the milk receiving platform. No equipment is required, but the milk grader must have good sense of sight, smell and taste. The result of the test is obtained instantly, and the cost of the test are low. Milk which cannot be adequately judged organoleptically must be subjected to other more sensitive and objective tests.

Procedure

- Open a can of milk.
- Immediately smell the milk.
- Observe the appearance of the milk.
- If still unable to make a clear judgement, taste the milk, but do not swallow it. Spit the milk sample into a bucket provided for that purpose or into a drain basin, flush with water.

Judgement

Abnormal smell and taste may be caused by

- Atmospheric taint (e.g. barny/cowy odour).
- Physiological taints (hormonal imbalance, cows in late lactation- spontaneous rancidity).
- Bacterial taints.
- Chemical taints or discolouring.
- Advanced acidification (pH < 6.4).

Estimation of phosphatase

Alkaline Phosphatase is an enzyme which is naturally present in milk, but is destroyed at a temperature just near to the pasteurization temperature. Alkaline Phosphatase test is used to indicate whether milk has been adequately pasteurised or whether it has been contaminated with raw milk after pasteurisation. This test is based on the principle that the alkaline phosphatase enzyme in raw milk liberates phenol from a disodium para-nitro phenyl phosphate and forms a yellow coloured complex at alkaline pH (Scharer, 1943). The intensity of yellow colour produced is proportional to the activity of the enzyme. The colour intensity is measured by direct comparison with standard colour discs in a Lovibond comparator. The test is not applicable to sour milk and milk preserved with chemical preservatives.

Procedure

Pipette 5 ml of buffer substrate into a clean, dry test tube

followed by 1 ml of the milk to be tested. Stopper the tube, mix by inversion and place in the water-bath. At the same time place in the water-bath a control tube containing 5 ml of the buffer substrate and 1 ml of boiled milk of the same kind as that under test that is pasteurized homogenized, low fat.. After 2 hours, remove the tubes from the bath, invert each and read the colour developed using the comparator and special disc, the tube containing the boiled milk control being placed on the left of the stand and the tube containing the sample under test on the right. Record readings which lie between two standard colour discs by adding a plus (+) or minus (-) sign to the figure of the nearest standard.

Note
If artificial light is needed when taking these readings, an approved 'day light' source of illumination must be used.

Test for adultrants

Table 1

Constituent	Experiment	Observation
Sugar	Take 5 mL milk sample in a test tube. Add 1 mL conc. HCl and 0.1 g resorcinol solution. Place the test tube in water bath for 5 min.	Appearance of red color indicates he presence of added sugar.
Starch	Take 3 mL sample in a test tube. After boiling it thoroughly, cool it to room temperature. Add 1 drop of 1% iodine solution.	Appearance of blue color indicates he presence of starch.
Glucose	Take 1 ml of milk sample in a test tube. Add 1 ml of modified Barfoed's reagent. Heat the mixture for exact 3 min in a boiling water bath. Rapidly cool under tap water. Add one ml of phosphomolybdic acid reagent to the turbid solution.	Immediate appearance of deep blue color indicates the presence of glucose.
Common salt	Take 5 ml of milk sample into a test tube. Add 1 ml of 0.1 N silver nitrate solution. Mix the content thoroughly and add 0.5 ml of 10% potassium chromate solution.	Appearance of yellow color indicates the presence of added salts, whereas, brick red color indicates the milk free from added salt.

Microbiological analysis of milk
Clot on Boiling (C.O.B) Test
The test was conducted according to the procedure given in IST (1960).

Procedure
This is a quick test to determine the developed acidity and the suitability of milk for processing. Five milliliters of the sample was taken in the test tube placed in a boiling water-bath and held for about 5 minutes. The sample was smelt for any acidic flavour. The tube was removed and rotated in an almost horizontal position and examined the film of milk or side of the test-tube for any precipitated particles. The formation of clot was indicative of a positive test. Milk which gave a positive COB test had acidity generally above 0.17 percent (as lactic acid) and was considered as not suitable for distribution as liquid milk for processing.

Methylene blue reduction test
This test was carried out by ISI (1960). Nhen methylene blue is mixed with contaminated milk, the methylene blue loses its colour (becomes reduced). This is the basis for the reductase test.

Procedure
Ten milliliters of thoroughly well mixed milk was taken in a sterile test tube, one milliliter of methylene blue was added to the above tubes. Then the tubes were sealed with a sterile rubber stopper with a sterile forceps and were slowly inverted three times to mix the dye with the milk and immediately the tube was placed in a water bath at 37.5°C. The level of the water in the water bath should be above the level of the milk in the tubes. Every half an hour the tubes were observed and those which had decolourized were removed from the bath and the time taken for reduction was noted. Those which had not decolourized were inverted once and replaced in the water bath and those which had shown partial decolourization were not inverted and placed in the water bath again. The tube without addition of methylene blue also was kept as a control. The reduction time of milk is roughly proportional to the bacterial count of milk.

Determination of total microbial population
The total microbial population was carried out according to the method described in Standard methods for the examination of dairy products (API-IA, 1978). Nutrient agar medium was used for the enumeration of bacterial population.

Dilution
Three or more test tubes containing exactly 9 milliliter of 0.9 percent sterile saline solution were arranged in a test tube rack. These are called dilution blanks. One milliliter of thoroughly mixed milk sample was pipetted by means of a sterile pipette and transferred to the first tube of the dilution blank. All the transfers were done under aseptic conditions. Care was taken to see that only the tip of the pipette was inserted into the liquid. In order to rinse the pipette the contents of the pipette was raised and lowered six times and finally allowed about three seconds to drain and the remaining contents were blown out. All these manipulations were carried out under aseptic condition.

Plating Technique
One milliliter of mixed sample was transferred to the test tube containing 9 milliliter of diluent. From this dilution serial dilutions were made.One milliliter of 10 dilution was poured into the pre-sterilized petriplates and then 15-20 milliliter, well mixed and previously cooled nutrient agar medium was poured into the petriplates. The plates were rotated for the contents to mix well. All aseptic precautions were taken till

inoculations were completed. After setting of the medium, the plates were inverted and kept in the incubator at 37°C for 24-48 hrs. Then the colonies were counted using colony counter.

Anti-microbial activity

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Similarly to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested

Results & Discussion

In spite of the remarkable developments in Indian milk industry there are still great difficulties with regard o quality as well as quantity of milk

Nutritional Status

Milk Fat

Lipids are essential constituents of all living tissues. They are vital components of brain and nerve cells and are essential to many physiological processes. They play an important role in both human diet and nutrition since it serves as a potential source of energy

Milk fat is the most valuable of all milk components. Milk lipids rate high for their pleasing flavour which is not duplicated in other types of food. They improve palatability by contributing to the texture and flavour. They also add to the satiety value since fat stay in the stomach longer than carbohydrates and proteins do. The range of fat percentage in the present study was 1.5 to 6, which was much Fair to the values given for cow's milk The fat percentage values are 4.3%, 4.5%, 6%,3%,1.5% for raw milk, SM,FCM,TM & DTM respectively

Biochemical Status

pH

The pH is defined as negative logarithm of hydrogen ion Concentration. This is a measure of the amount of active hydrogen (H) or hydroxyl (OH) ions present in a solution. For a perishable food product of exceptional nutritive value like milk, its hydrogen-ion concentration is an important property governing stability and behavior under various conditions. Therefore the pH indications are used in the dairy industry for various purposes beginning from the production of milk till its disposal either for liquid consumption or in the manufacture of products of long keeping quality disposal of dairy effluents and quality of brine used for refrigeration

In the present study the pH range of milk are 6.7, 7.1, 7.1, 7.1 & 7.2 respectively for raw milk, SM, FCM, TM, DTM. Value 6.7 indicates that the milk is Mastitic

Specific Gravity

The Specific gravity of Milk is measured at 20 degrees and it is normally 1,028 to 1,033 kg / litre. The specific gravity depends on the protein & fat content of the milk. The present study reveals the specific gravity of different milk sources as 1,032, 1,037, 1,023, 1.002, and 0.8773 respectively

Moisture and Total Solid

Milk is a natural liquid food containing a high percentage *of* water. Milk is actually a concentrated food designed to produce rapid growth in young mammals and contains more solid material than many of our other common foods. If milk is without water, it seems highly viscous and so hard for the calf to snipple. Hence Moisture plays an important role in quality assurance of Milk which is 88%, 73%, 72%, 73%, 69% respectively for raw milk, SM, FCM, TM, DTM respectively in our present study.

TDS refers Total Dissolved Solids which means the suspended solid components of milk such as protein, fat and others. TDS was found to be 11.8 ppm, 13.3 ppm, 13.7 ppm, 13.2 ppm, 12.7 ppm respectively for different milk samples viz raw milk, SM, FCM, TM, and DTM

Note: Higher moisture contents with the simultaneous reduction in the total solids in any milk sample might be attributed to the adulteration by the unscrupulous vendors.

Acidity

The acidity of fresh milk is due to certain constituents of milk like phosphates, proteins and to a slight degree by the presence of carbon dioxide and citrates. Generally the acidity of milk is first detected by taste when pH drops to about. 6.0. When milk is freshly drawn from the cow, it shows an amphoteric reaction. As fresh milk contains no lactic acid, this acidity is apparent and is a measure of the amounts of alkali combines with the protein arid mineral salts *in* the milk. When bacterial fermentation takes place, lactic acid is formed and the acidity of milk is increased. The acidity determination is valuable to use as a guide in manufacturing operations and measuring the quality of dairy products. When fresh milk is titrated with a standard solution of alkali, its acidity is equivalent to 0.13 to 0.18 % of lactic acid. Acidity of milk samples of the present study showed the values of 0.12 and 0.14, as 0.12 for raw milk & 0.14 for the other four sources

Alcohols

Alcohols are the major by-products of carbohydrates when fermented. Lactose a carbohydrate, present in milk when ferments forms alcohol, which tends the milk sour. Hence Alcohol Test is the best index for checking the quality of milk. In the present Study Alcohol Test is Negative in all different kinds of milk sources.

Organ Test

It is the manual test performed with sensory organs viz with Nose & Tongue by Smelling & Tasting the sample. In this study the milk sources are found to be OK and fit for consumption.

Phosphatase Test

It is the Test performed to check how far the milk is pasteurized with the indication of an enzyme phosphatase in

it. In our study there is no trace of Phosphatase present in differ rent kinds of the sample taken for analysis

Test for adulteration

Adulteration is the process of adding same class of impurities that doesn't tend to change the quality of the food stuff and they are also added to enhance the best appearance of the food stuff. As far in dairy processing components such as Sugar, Starch, Salt, Urea & Glucose are added as adulterants to enhance the appearance & quality of milk. In our study no such components were detected

Microbial Status

Clot & Blot Test

It is the Test to assess the microbial load in the Milk sample. Due to microbial load milk clot's and tends sour, which is not suitable for consumption. In our study No Clots were recorded in different kinds of milk samples taken for analysis.

Methylene blue reduction test

Methylene Blue Reduction Test (MBR) is used to assess the quality of milk and indirectly show the microbial load. Milk of poor quality would have lower MBR value indicating a high growth rate of microorganisms causing quick acidity and prominent flavor development in milk. The milk would be satisfactory if it had more than *240* minutes. In our present study the MBRT Time is 120 min for raw milk and 360 min for other samples respectively, as the bacterial population of milk sample increased there was simultaneous reduction in MBR.

Total count of microbes

Prevalence of bacterial pathogens in raw milk is obviously high due to, Livestock farms showing the poor type of sanitary conditions of milking and utensils cleaning prevailed. Raw milk is regarded as very good, good, fair and poor when it showed the bacteriological population not exceeding 2,00,000, 2,00,000 to 10,00,000, 10,00,000 to 50,00,000 and above 50,00,000 per gram. In our present study the total count of Microbe in raw milk is 1.8×10^3 cfu per milliliter of sample; other sample shows no growth of microbe

Note: Normally, pH of milk would drop due to acid production by the increased microbial activity. This phenomenon was observed in the present study too.

Antimicrobial Activity

There is a diffusion seen for *E.coli* (Raw Milk) in Well diffusion method hence we can conclude that raw milk possess Mild Anti microbial Activity for *E.coli* since the width of diffusion is below 1mm

Table 2

Sl. No	Status	Parameter	Raw Milk	Processed Milk			
				SM	FCM	TM	DTM
1.	Nutritional Status	Fat	4.3%	4.5%	6.0%	3.0%	1.5%
2.	Biochemical Status	pH	6.7	7.1	7.1	7.1	7.2
3.		Specific Gravity	1.032 gm/lit	1.037 gm/lit	1.023 gm/lit	1.002 gm/lit	0.8773 gm/lit
4.		Moisture	88%	73%	72%	73%	69%
5.		TDS	11.8 ppm	13.3 ppm	13.7 ppm	13.2 ppm	12.7 ppm
6.		Acidity	0.140	0.140	0.140	0.140	0.140
7.		Alcohol	-	-	-	-	-
8		Organ Test	OK	OK	OK	OK	OK
9.		Phosphatase	+	-	-	-	-
10	Adulterant	Sugar	-	-	-	-	-
11.		Starch	-	-	-	-	-
12.		Salt	-	-	-	-	-
13.		Urea	-	-	-	-	-
14.		Glucose	-	-	-	-	-
15.	Microbial Status	COBT	No clot	No clot	No clot	No clot	No clot
16.		MBRT	2 Hrs	6 Hrs	6 Hrs	6 Hrs	6 Hrs
17.		Total Count	1.8×10^3 cfu/mm	Nil	Nil	Nil	Nil
18.	Antimicrobial Activity	*Coli form Count*	Seen	Nil	Nil	Nil	Nil

Note: It is scientifically proven that Milk Sugar Lactose cannot be digested by the enzymes of our body it can only be done by E.Coli present in colon; also it inhibits E.coli which is primarily important as said earlier due to the presence of Lactoferritin an Iron rich Protein.

Fig 1

Fig 2

Conclusion & Recommendations

Fat content of milk is considerably good

However, no coliforms are found to be present in the processed milk in 1 / 100 dilutions.

On the basis of overall assessment of biochemical and microbiological qualities of milk sold may be classified as 'Good for Consumption'.

Consumer awareness must be created regarding the microbiological quality of milk

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