STUDENT STUDY PROJECT (2016-2017)

Name of the Topic: Qualitative analysis of Milk

Under the Guidance of

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INTRODUCTION

Milk is the nutrient fluid produced by the mammary glands of female mammals (Including monotremes).The female ability to produce milk is one of the defining characteristics of mammals. It provides the primary source of nutrition fornewborns before they are bale to digest more-diverse foods. the early lactation milk is known as colostrums , and carries the mother's antibodies to the baby. it can reduce the risk of many diseases in both the mother and baby.

COMPONENTS OF MILK

- The exact components of milk vary by species, but it contains significant amounts of saturated fat proteins and calcium
- These amounts are not large comparison to other foods rich in them, including coconuts fish and kale respectively, because milk is predominantly composed of water.
- Aquatic mammals, such as seals and whales, produce milk that is very rich in fats and other solid nutrients when compared with land mammals" milk.

The term milk is also used for non-animal substitutes such as soy milk ,rice, milk, almond milk, and coconut milk, and even the reg8igatated substance pigeons feed their young, called crqp milk, which bears little resemblance to mammalian milk.

MATERIALS AND METHODS

Methods of Investigation

1. Most probable Number Method
2. Gram staining Method
3. Methylene Blue Reductase Method

Scope of Investigation :

To test the quality of Milk.

1. MPN METHOD [Most probable Number Method]

Introduction:

Coliform bacteria are indicators of fecal contamination. The estimation of Coliform bacteria is carried out sequentially in three stages i) Presumptive Test, ii)Confirmed Test and iii) Completed Test. Coliform bacteria are aerobic or facultatively anaerobic, Gram _ve rod shaped, non-endospore forming, capable of fermenting lactose with the production of acid and gas within 24 hours of incubation at 370 C.

Materials:

Medium: Lactose Broth (S.S)

Beef extract	3.0
Peptone	5.0
Lactose	5.0

Eosin – Methylene Blue (E M B) Agar

Peptone	10.0
Lactose	5.0
Dipotassium phosphate	2.0
Agar	13.5
Eosin	0.4
Methylene blue	0.065

i.Presumptive Test:

This test is used to detect and estimate coliform bacterial population in milk sample. The test is known as presumptive because of the development of positive result in Mac Conkey broth inoculated with Milk. In this test, known volume of Milk is added to lactose fermentation tubes and production of acid and gas from the fermentation of lactose is a positive test for the Caliform bacteria. A statistical method is used to estimate the presence of populatin of Coliforms expressed as the most probable number (MPN). A count of number of lactose fermentation tubes showing production of gas following incubation period is taken and MPN is found by matching the results with those provided in the statistical table.

ii. Confirmed Coliform Test:

This test is used to confirm the presence of coliforms in Milk sample showing positive or doubtful presumptive test. In the confirmed test, the samples from the post presumptive lactose broth tubes are streaked onto a selective differential medium for coliforms. The medium commonly used is eosin methylene blue (EMB) agar which is a selective medium as methylene blue inhibites the growth of gram (+)ve bacteria. The medium is also differential as it gives coloured colonies of lactose fermenting bacteria due to formation of complex. Non-lactose fermenters produce colourless colonies on EMB agar.

Requirements:

EMB agar plates, 24 hours old positive lactose broth cultures, inoculation loop.

Procedure:

Streal EMB agar plates with (+)ve 24 hours lactose broth culture with a sterile inoculation loop and incubate the plate for 24 – 48 hours at 37C in an inverted position. Examine the inoculated plate for the presence or absence of E. coli colonies.

TABLE:

Most probable Number **(MPN)** of coliform bacteria present in 100ml of milk for various combinations of Positive and Negative results when three each of 10 ml, 1 ml and three 0.1 ml portions are used.

Number of tubes giving positive reactions			**MPN Index Per 100ml**
3 of 10 ml each	**3 0f 1 ml each**	**3 of 0.1 ml each**	
0	0	1	3
0	1	0	3
1	0	0	4
1	0	1	7
1	1	0	7
1	1	1	11
1	2	0	11
2	0	0	9
2	0	1	14
2	1	0	15
2	1	1	20
2	2	0	21
2	2	2	28
3	0	0	23
3	0	1	39
3	0	2	64
3	1	0	43
3	1	1	75
3	1	2	120
3	2	0	93
3	2	1	150
3	2	2	210
3	3	0	240
3	3	1	460
3	3	1	1100
3	3	3	2400

2. GRAM STAINING

AIM: To identify the given milk sample by gram staining.

Principle : Gram staining is also called as differential staining as it differentiates Gram +ve & _ ve bacteria. There two stains are used . Crystal violet as primary stain & saffaranin as counter stain After treating with crystal violet grams iodine is added. Thus crystal violet complex is formed .Complex is formed. The slide is heated with decolorized i.e alcohol & finally with saffaranin.

Gram +ve bacteria has thick peptide glycan layers after dehydration wit decolouriser. These pore size of cell wall &cell membrane decreases. Thu crystal violet primary stain. But Gram '_ve' bacteria have then peptiaoglyco and an outer membrane which is rich in lipids. After treating with alcoho their cell wall pore size increases due to lipid extract, leaking out cryst violet iodine complex thus they take up counter stain & appear pink i colour.

Requirements:

Milk sample , crystal violet _ grams iodine, 95 % alcohol, saffaranin. DH2, spirit lamp, inoculation loop, glass slide emersion oil, micro scope.

GRAM STAINING

Prepare milk smear

Air dried & heat fix it

Add Primary stain
(Crystal violet or methylene blue)

Slide was washed with DH2O

Treat the slide with gram iodine
for 1 min. & wash with DH2O

Add colouriser (90% alcohol)
Wash with distilled water.

Add colour staining

Wash with slide with Dh2O air dry
& observe under microscope.

3. METHYLENE BLUE REDUCTASE TEST

Aim: To determine the quality of milk sample.

Principle: Bacteria present in the milk utilize oxygen which is present in small amount producing a reduced environment. The reductase test is based upon the oxidation reduction test is based upon the oxidation reduction test is based upon the oxidation reduction activities of the bacteria present in the milk. In this test, methylene blue, which is colour sensitive to oxygen concentration is added to the milk, the indicator is blue in oxidized state and white in reduced state, the more the bacteria present the faster the reduction.

Reduction within 30 min.	-	very poor quality.
Reduction between 1 ½ - 2 hours	-	poor quality
Reduction between 2 – 6 hours	-	fair quality
Reduction between 6 – 8 hours	-	good quality.

Requirement:

1) Milk Sample (Raw1, Raw2, Skimmed & Pasteurised)
2) Methylene Blue Solution
3) Test tubes
4) Cotton plugs
5) Pippettes etc.

Requirements:

- Raw milk
- 9ml single strength lactose fermentation broth tubes with Durham tubes (6 no.),
- 20 ml double lactose strength fermentation broth tubes with Durham tubes (3 no.),
- sterile pipettes (one each of 10ml, 1 ml and 0.1ml).

Procedure:

1) Take milk sample in glass bottle or conical flask.
2) Label 3 single strength lactose broth tubes as 0.1, another 3 tubes as 1.0 and 3 double strength broth tubes as 10.
3) Inoculate aseptically '0.1' tubes with 0.1 ml sample with the help of 0.1 ml sterile pipette.
4) Inoculate aseptically '1' labeled tubes with 1 ml sample using 1. ml sterile pipette.
5) Inoculate aseptically '10' labeled tubes with 10 ml sample using 10 ml sterile pipette.
6) In cubate all the 9 tubes at 37 C for 24 hours. Observe for production of acid and gas.
7) Read the MPN index / 100 ml from a standard table.

Observations and Results:

Appearance of typical coliform colonies (E.coli) with dark centres and metallic sheen is a confirmed test for the presence of coliforms and indicates that the Milk is non-potable.

iii. Completed Coliform Test:

Completed Test is used as a further confirmatory test for the presence of E.coli in water sample. In this test lactose positive colonies from EMB agar are isolated and inoculated into a lactose broth tube and streaked on a nutrient agar plate with the help of loop. A|cid and gas production in lactose broth, confirms the presence of E. coli in a given sample.

Requirements:

Twenty four hours old culture of coliform EMB agar cultures, lactose fermentation broth tubes, nutrient agar slants and gram stain reagents.

Procedure:

1) Inoculate fermentation broth tubes with isolated coliform colonies of EMB agar plates using inoculating loop.
2) Streak nutrient agar slant with colony from EMB agar plate using inoculating loop.
3) Incubate the inoculated broth tubes and slants at 37 C for 24 hours.
4) Stain the bacteria with the help of Gram stain.

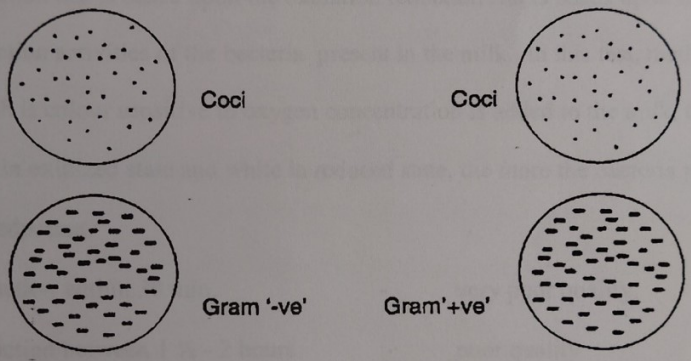
Procedure:

A smear was prepared using the given bacterial culture and heat fixed.

- The smear was flooded crystal violet strain for 1 – 2 min, then slide was washed with DH2O.
- 1 to 2 drops of gram iodine was added onto smear & allow it to act for 1 min, then wash with DH2O.
- The smear was added with decolourising agent (95% alcohol) till the violet colour washed off the slide was then washed with DH2O
- Finally, the smear was counter stain with saffaranin for 1 min. & slide was again washed with DH2O to remove excess of stain.
- Now, slide was block dried & observed under oil emersion.

Observation:

Gram '+ve' bacteria take up primary stain & appeal purple in colour gram '-ve' bacteria take up counter stain and appear pink in colour.



Results:

Gram '-ve bacilli bacteria with purple stain.

Procedure:

- 10 ml. of each milk sample was taken in a sterile test tube using the pipettes.
- 1 ml. of metheline blue dye was added to all the test tubes.
- Tubes were shaken and placed in water bath at 37 C.
- Tubes were allowed to stand and observed periodically for disappearance of blue colour.
- Time taken for the disappearance of blue colour was recorded.

Discussion:

Milk gets contaminated when exposed to air and also due to poor handling. It requires an oxidation reduction potential due to microbial growth. As microorganisms grow in milk. They cause a shift charge in the oxidation reduction potential by reducing the quality of oxygen. This shift can be detected by various dyes like Methylene blue, reszurine, which undergoes colour change when reduced or oxidized microbial reduction test depends on the fact that dye is blue when oxidized and turns colour less when reduced. Thus the rate of reduction is directly proportional to the no. of microorganisms present in it.

Result:

In Raw Milk sample – 1, colour change was seen in 30 min. Hence the quality of mik is very poor.

In Raw Milk sample – 2, colour change was seen in 2 hours. Hence the quality of milk is poor.

In skimmed milk sample – 3, colour change was seen in 4 hours. Hence the quality of milk is fair.

In pasteurized milk sample – 4, colour change was seen in 6 hours. Hence the quality of milk is good.

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OBSERVATIONS AND RESULTS

1.Most Probable Number Method :

- Appearance of typical coliform colonies (E.coli) with dark centres and metallic sheen is a confirmed test for the presence of coliforms and indicates that the milk is no potable.

2.Gram Staining Method:

- Gram '+ve' bacteria take up primary stain & appeal purple in colour gram '-ve' bacteria take up counter stain and appear pink in colour.
- **RESULT:** Gram '-ve' bacilli bacteria with purple stain

3.Methylene Blue Reductase Method:

Result:

In Raw Milk Sample – 1, colour change was seen in 30 min. Hence the quality of milk is very poor.

In Raw Milk sample – 2, colour change was seen in 2 hours. Hence the quality of milk is poor.

In skimmed milk sample – 3, colour change was seen in 4 hours. Hence the quality of milk is fair.

In pasteurized milk sample – 4, colour change was seen in 6 hours. Hence the quality of milk is good.

THE BENEFITS OF MILK

Milk is considered as a complete and ideal food and it contains most of the proximate principles of a well balanced diet. Milk of various mammals is used for food but cow's milk is being used throughout the world for feeding infants and as a supplement to the diets of the children and adults. The other animal's milk used are buffalo, goat , sheep, and camels. This nutrient packed drink is given to patient even during critical stage. Various milk products such as curd, butter milk, ghee, cheese, pannier, khoya, rabri. etc are used commonly in our food preparations. This time tested nutritious drink is being criticized by few local people to the extent that milk is equated to poison.