Sampling of Milk for Analysis

Aim:

To become familiar with the different procedures to be followed in collecting samples of milk and milk products for analysis.

Principles:

The sampling procedures differ according to the nature of the material and the purpose for which it is needed. The sample should be such that it should be truly representative of the bulk. The different procedures to be followed in collecting a sample of milk or milk products are given below:

Sampling of milk from a single container.

The operations involved are mixing and then immediate taking of this sample. Mixing can be done either by using a plunger which should be moved up and down the milk vigorously for about ten times. If it is in a small container it should be poured from one vessel to the other and shaking it. Care should be taken to avoid fat separation.

If samples of milk of individual cows are needed, it may be done in the weighing room itself by following the above procedure:

Collecting a composite sample from a number of containers:

1. Pour all the milk from the different containers into a vat if feasible and then take a single sample as above.

2. If the cans are the same diameter the following procedures can be applied.

   a. Mix the milk in each of the cans with the help of a plunger, then equalize the level of milk in all these cans and withdraw equal quantities from them and put together.

       OR

   b. The tube method of sampling can also be applied in the above instance. Mix the milk with a plunger. Introduce a sampling tube of uniform bore, which is opened at one end, and having provision for closing the other end. Close the tube and withdraw it. A column of milk equal in height with the height of milk in the can will be remaining in the tube. Transfer this to a container. Repeat the process with the other remaining cans. Collect together in the sample bottle.

Composite Sample of Individual Animals:

For a number of days or herd sample required to study the composition of milk scattered over a period of days is done as follows. Transfer
quantities of milk proportional to the total bulk at each milking. Add formalin at the rate of 0.1 ml for 25 ml of milk.

To draw samples from bulk units containing uniform quantities of milk the procedure to be adopted is as follows:

<table>
<thead>
<tr>
<th>Total No. of Units</th>
<th>No. of Units to be selected:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6–20</td>
<td>3</td>
</tr>
<tr>
<td>61–100</td>
<td>4</td>
</tr>
<tr>
<td>Over 100</td>
<td>5 plus one for each additional 100 units or fraction.</td>
</tr>
</tbody>
</table>

**Sampling from storage tanks, rail and road milk tankers:**

Mix the milk thoroughly using a large plunger or mechanical agitator or by bubbling compressed air. Insert the plunger through the manhole and move it forward, downward and backward and each time bring it to the surface. This should be done for 15 minutes. Transfer 100–250 ml of the mixed milk by a suitable dipper to a sample bottle. A composite milk sample for fat test is prepared over a period. The total volume of individual composite milk sample should not be less than 150 ml. It is collected during the prescribed period as proportionate quantities of milk from the supply. As preservative 0.1 ml of 36% Formaldehyde (Formalin) for each 25 ml of milk sample is used. The bottle should be tightly stoppered to prevent evaporation and kept away from light. It should be tested on the same day as the last portion of milk is collected into the sample bottle. After each addition of milk the contents of the sample bottle are mixed by gentle rotation of the bottle.

The sample bottle used should be wide mouthed with slopping sides and should have well fitting caps. The sample bottle should be of such a size that when the required quantity of sample is put into it there should not be much space after putting the stopper. Only rubber stopper should be used. Each bottle should be properly labelled for its contents. When mercuric chloride is used for freezing point determination the bottle should be labelled clearly as “Poison”.

Mercuric chloride is to be added at 0.5 gm for each 250 ml of milk.
Treatment of Milk on Arrival at the Laboratory before Analysis:

Warm the sample in the bottle to about 40 degrees Celsius in a water bath and mix thoroughly. Cool to 26 - 28 degrees Celsius. Leave aside the sample for about 4 minutes. After that mix the sample inverting the bottle 3 - 4 times and start analysis.

Determination of Acidity of Milk.

Aim:

To determine the natural acidity of milk.

Apparatus:

Burette 50x1/10 ml with soda line guard tube; porcelain dish-white flat-bottomed of approximately 100 ml capacity; stirring rods; pipette- 10 ml; pipette - 1 ml.

Reagents:

N/9 Sodium hydroxide solution, 0.5% phenolphthalein indicator 0.005% with Rosaniline acetate solution.

Principles:

Natural acidity in milk is due to its constituents such as casein, albumin, citrates, phosphorus and carbon dioxide. This acidity can be measured by titrating milk against a standard alkali using an indicator like phenolphthalein and is expressed in terms of lactic acid.

Procedure:

1. Thoroughly mix the sample of milk avoiding incorporation of air bubbles and transfer 10 ml with the pipette to each of the two porcelain basins.
2. Prepare a blank by adding 1 ml of rosaline acetate solution to 10 ml of milk in one of the porcelain basins.
3. Add 1 ml of phenolphthalein indicator to the other 10 ml of milk.
4. Rapidly add 1 ml of N/9 NaOH solution to milk containing phenolphthalein and continue to add drop by drop until by comparison the colour matches the pink tint of the blank.
5. Repeat the experiment to get concordant values.
**Observations:**

*Record the values as shown below:*

<table>
<thead>
<tr>
<th>Burette reading</th>
<th>Titration No.1</th>
<th>Titration No.2</th>
<th>Titration No.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final reading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial reading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume delivered in milk</td>
<td></td>
<td></td>
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</tbody>
</table>

N.B: **Acidity Test:**

For N/10 NaOH = Titre value x 0.09 = % acidity  
For N/9 NaOH = Titre value/10 = % acidity.

**Conclusion:**
Natural acidity of the given sample expressed as percentage of lactic acid is:-

**Title:**
To determine the total acidity (Natural + developed) of milk.

**Apparatus:** Same as above (i.e. previous exercise)

**Reagents:** Same as previous exercise.

**Principle:** Fresh milk on keeping at room temperature for sometime develops acidity due to bacterial action. This along with the natural acidity could be measured by titrating a known volume of milk with a standard alkali to the end point of an indicator like phenolphthalein.

**Procedure:**
Keep the bulk sample of milk used in experiment 1(3) for about 4 hours at room temperature to get developed acidity. Estimate the acidity of the sample as in 1 (3) A.

**Observations:** As previous exercise.

**Calculations:** As previous exercise.

**Conclusion:**
The acidity of the given sample is expressed as percentage of lactic acid =

Percentage of Natural acidity is expressed as lactic acid. Therefore, the percentage of developed acidity in terms of lactic acid = Total acidity - Natural acidity

Note: 1. Thorough mixing should be done during titration

2. Titration should be carried out within 20 seconds.

3. Titration shall be made under diffused daylight or under illumination
Sampling of milk and milk products

from a daylight lamp complying with B.S. 950.

Preparation of Gerber Sulphuric Acid.

Aim.
To prepare Gerber Sulphuric acid from concentrated sulphuric acid by dilution with water.

Apparatus:
Hydrometer range 1.800 to 2000, measuring cylinder, round bottom flask.

Preparation:
Take required volume of water in a pyrex flask (generally 100 ml of water is required for 900 ml of sulphuric acid conc.) kept in a basin of ice cold water. Carefully add the commercial sulphuric acid in small quantities at a time keeping the container sufficiently cold. Mix gently. Observe the following precautions while performing the above experiment.

1. Sulphuric acid is very corrosive. Handle it with care.
2. Add Acid to Water. Add small quantities of acid to water at a time and cool the mixture by stirring. "NEVER ADD WATER TO ACID"
3. Use heat resistant flask for dilution.

After cooling check the specific gravity of Gerber acid with hydrometer and if necessary adjust the Gerber acid to the correct specific gravity with addition of water or acid taking same precaution as before till specific gravity is in the range of 1.815 to 1.820 at 27 degrees Celsius. Store in a glass stoppered bottle to avoid absorption of water. Record the following observations:

a) Specific gravity of Conc. sulphuric acid.
b) Volume of concentrated sulphuric acid taken for dilution.
c) Volume of water used for dilution,
d) Specific gravity of Gerber acid.
e) Volume of Gerber acid obtained.
f) Result of fat test- Satisfactory/not satisfactory.

Testing of Amyl Alcohol for Gerber Fat Test.

Apparatus: Boiling point apparatus with a centigrade thermometer 0 °C to 200 °C. Test tubes, test tube stand, Westphal balance or hydrometer to measure density and apparatus to carry out Gerber fat test.

Reagents:
Sulphuric acid 97 percent (density 1.838/ml at 27 degrees centigrade), Conc. HCL.

**Principle:**

If proper quality of amyl alcohol is not used the separation of fat column in the test is not proper. In addition wrong results may be contaminated. The amyl alcohol to be used in Gerber test shall be a clear, colourless or almost colourless liquid free from water, furfural, acid and fatty matter and shall consist principally of Iso amyl alcohol. The density shall be 0.805 g/ml at 27 degrees Celsius. Boiling range: It should begin to boil at 128 - 129 degrees Celsius at 760 mm pressure and 95 percent of the liquid shall distil between 130 - 132 degrees Celsius.

**Procedure:**

*Carry out the following tests:*

1. Density- Find out the density by use of Wesphal balance or hydrometer.
2. Determine the boiling point of the liquid.
3. Test for absence of furfural and other organic impurities as follows: Take 5 ml of amyl alcohol in a test tube and add to it 5 ml sulphuric acid (97%). Observe the colour. Amyl alcohol shall not show more than a yellow or light brown colour.
4. Test for absence of fatty matter. Carry out a blank fat test using distilled water instead of milk and observe the butyrometer reading. If any fat separation is observed, it is due to some fatty matter present as an impurity in amyl alcohol.

**Determination of Fat in Milk by Gerber Method:**

**Aim:** To determine the percentage of fat in milk by Gerber Method.

**Apparatus:**

Gerber centrifuge machine, Gerber butyrometer for milk (0 - 10% scale with 0.1 percent mark), hot water bath maintained at 65 plus or minus 2 °C, 10 ml automatic measure for acid, 1 ml automatic measure for amyl alcohol, 10.75 ml pipette, butyrometer stoppers, butyrometer stand, cotton wool.

**Reagents:**

Gerber sulphuric acid density 1.807 to 1.812 g/ml at 27 degrees centigrade corresponding with a concentration of sulphuric acid from 90 - 91 % by weight. Amyl alcohol 95% of clear, colourless liquid shall distil between 130 to 132 degrees centigrade, density 0.803 to 0.805 g/ml at 27 degrees centigrade.
Principle:

When a definite quantity of sulphuric acid and amyl alcohol are added to a definite volume of milk, the proteins will be dissolved and the fat globules will be set free which remain in liquid state due to heat produced by the acid. On centrifugation fat being lighter will be separated on top of the solution.

Procedure:

1. Take 10 ml of Gerber sulphuric acid from automatic measure into the butyrometer.

2. Pipette out 10.75 ml of the well mixed sample of milk and transfer it to the butyrometer carefully without allowing it to mix with the acid. This is done by allowing the jet of milk from the pipette to hit the inside wall of the butyrometer by holding the pipette in slanting manner and resting the tip end on the mouth of the butyrometer.

3. With the help of automatic pipette add 1 ml of amyl alcohol to the above butyrometer.

4. Tighten the stopper and mix the content by shaking the butyrometer at 45 degree angle until all the curd has been dissolved.

5. Keep the butyrometer in the water bath at 65 degrees centigrade plus or minus 2 °C for 5 minutes.

6. Place the butyrometer in the centrifuge and balance the machine. Centrifuge for 5 minutes (1000 - 1200) r.p.m.

7. After centrifuging, temper the butyrometer in the water bath at 65 degrees centigrade plus or minus 2 degrees centigrade for 5 minutes.

8. Adjust the fat column within the scale on butyrometer and take the reading.

Observation: Record the fat percentage.

Determination of Specific Gravity of Milk Using Lactometer and Calculation of Total Solids Not Fat and Total Solids in Milk Using Richmond’s Formula:

Aim.

To determine the density and specific gravity of milk using density hydrometer and specific gravity lactometer and to calculate the total solids and solids-not-fat in milk using Richmond's formula.

Apparatus:

Specific gravity lactometer, lactometer jar and thermometer. Other apparatus are same as that of Gerber fat test.

Reagents: Same as that of Gerber fat test.
**Procedure:**

1. Warm the milk sample to 40°C and maintain this temperature for 5 minutes.
2. Remove the sample bottle and mix the contents by rotating and inverting the bottle, taking care to avoid the formation of air bubbles and froth.
3. Cool the sample approximately near to the calibrated temperature of the lactometer. The temperature of milk at the time of taking lactometer reading should be within the range shown in the correction tables.
4. Invert the sample bottle two or three times, pour enough milk into the glass cylinder taking care to avoid the formation of air bubbles, so that some milk overflows when the lactometer is inserted.
5. Insert the lactometer gently to wet the stem not more than a short length, about 3 mm beyond the position of equilibrium. The lactometer should float freely and not touch the sides of the cylinder.
6. Allow the lactometer to remain steady in the milk. Take the reading within about 30 seconds. Note the reading of the lactometer corresponding to the top of the meniscus on the stem without the error of parallax.
7. Note the temperature of the milk.

**B.** Determine the fat percentage of the sample as under Exercise 2.

**Observation:**

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Observed lactometer reading</th>
<th>Temperature</th>
<th>Fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Calculate the total solids and solids not fat.

1. Obtain the corrected lactometer reading by applying approximate correction factor by referring to the approximate temperature correction chart.

2. Calculate the total solids and solids not fat using suitable Richmond’s formula for the type of lactometer used, as given below.

\[
\text{S.N.F \%} = \frac{\text{CLR}}{4} + 0.21F + 0.66
\]

(Temp. 84 degrees Fahrenheit)

\[
\text{T.S.} = \text{S.N.F.} + F.
\]