

ANALYSIS OF MARKET SAMPLES OF MILK AND MILK PRODUCTS

9.1 Introduction

Milk is an extremely complex biological fluid with scores of nutrients contained in a fluid with characteristics of three physical phases: a dilute emulsion, colloidal dispersion and a solution. The emulsion can be broken by low speed centrifugation and the milk separates into lipid and aqueous phases or compartments, each with a characteristic composition. The chemical makeup of milk and its physico-chemical behavior provide scientific basis for process of milk and manufacture of products. Milk is valued commercially for two parameters namely fat and solids-not-fat (SNF). The SNF largely consists of proteins, lactose and minerals. The term total solid (TS) refers to the quantity of SNF plus fat present in milk. It may range from 12 to 16 percent, depending on its source. For cow milk, TS is 12 percent (3.5 % F and 8.5% SNF) while for buffalo milk it ranges between 15 and 16% (6-7% F and 9-10% SNF). Apart from species and breed related differences, certain other factors also influence the composition of milk. They are: individuality of animal, stage of milking, intervals of milking, completeness of milking, frequency of milking, irregularity of milking, portion of milking, different quarters of udder, lactation period, yield of milk, season, feed, nutritional level, environmental temperature, health status, age, weather, oestrus or heat gestation period, exercise, excitement and administration of drugs and hormones. In general, these variables tend to average out but show a seasonal pattern in the production of commercial milk used by dairy processors. This periodic fluctuation could have an important impact on properties of the finished products. However, variations in the concentration of an individual chemical entity are in certain ranges and the average values for these chemical constituents in milk provide useful information for several purposes:

- Nutritive values of milk and milk products
- Classification of milk as a basis for payment
- Detection of adulteration
- Breeding of milch animals for herd improvement
- Control of hygiene and health status of herd
- Production of specific product
- Processing effect on chemical quality
- Statutory requirement for meeting standards

So, for the above mentioned purposes, it is very necessary to go for the chemical analysis of milk and milk products. Before analysis, the sampling of milk and milk products shall be done by an experienced person as explained earlier in Lesson 7; Para 1.

9.2 Platform tests

Milk is a product of biological origin and is a suitable vehicle to accommodate any additive without apparent changes in its look. The quality of milk and its products depends upon quality of raw milk used in their manufacture, processing and handling conditions. It is therefore, necessary to check quality of raw milk to assess its suitability for processing, through various quick tests called “Platform Tests”. Moreover these tests are called platform tests because they are performed at the reception dock where milk is received and checked for its quality using various tests before either rejecting or accepting the milk supply for further processing.

9.2.1 Organoleptic test

Organoleptic test are used in all dairies and an experienced person can pick out bad samples with a high degree of accuracy. Judging the quality of milk by its taste and smell requires considerable skill which could only be acquired by practice.

9.2.1.1 Procedure

Remove lid from the can/tanker and observe for any extraneous matter present. Sniff the milk for any objectionable flavor that is un-natural of milk. Put small quantity (10 – 20 ml) on tongue and roll into mouth cavity for any off taste that is un-natural of milk and spill out. In case of doubt, subject the sample to other tests. Based on organoleptic evaluation accept or reject the milk.

9.2.2 Clot-on-boiling test (COB)

This is a quick test to determine developed acidity and to assess the suitability of milk for heat processing.

9.2.2.1 Procedure

Take 5.0 ml of milk in a 20 ml test tube using graduated pipette. Place the tube in boiling water bath for five minutes or hold the tube over a flame and allow the contents to boil. Formation of clots or flakes on the test tube wall indicates positive test. This further indicates that the milk has high developed acidity and is not suitable for heat processing.

9.2.3 Alcohol test

The alcohol test is used for rapid assessment of stability of milk to processing, particularly for condensing and sterilization. This test gives an indication of the

mineral balance of milk. The test aids in detecting abnormal milk such as colostrum, milk from the animal in the late lactation, milk from animal suffering from mastitis and milk in which mineral balance has been disturbed.

9.2.3.1 Procedure

Take 5.0 ml of milk in a test tube using graduated pipette. Add equal amount of ethyl alcohol (75% ethyl alcohol for cow milk and 68% ethyl alcohol for buffalo milk). Close the mouth of the test tube with thumb and mix the contents well by inverting the test tube several times. Formation of any flakes on the wall of the test tube indicates positive test.

9.2.4 Alcohol –alizarin test

The test is similar to the alcohol test and the incorporation of Alizarin helps to indicate the approximate percentage of acidity. So this test also indicates the suitability of milk for high heat treatment and to have idea about milk acidity without acidity test.

9.2.4.1 Procedure

Transfer 5.0 ml of milk in a test tube using graduated pipette. Add equal amount of alcohol-alizarine solution (0.2%). Mix the contents well by inverting the tube several times. Observe for formation of flakes and color of the contents. Match your results with following table

Table 9.1 Alcohol-alizarin test observations

Range of color	Presence of flakes	Approximate Acidity (%)
Pale red	Nil/no	0.16
Reddish brown	Small	0.20
Yellowish brown	Small	0.24
Brownish yellow	Large	0.28
Yellow	Large	0.36

9.2.4.1 Detection of neutralizers

Alkali in various forms like sodium carbonate, sodium bicarbonate, sodium hydroxide

and lime are used to neutralize developed acidity in milk. Detection of such neutralizers can be made by the following two tests. For details, refer Lesson 8.

9.3 Laboratory Tests

9.3.1 Acidity test

Normal acidity of milk is due to its constituents like casein, citrates, phosphates and CO₂. This acidity can be measured by titrating milk against a standard alkali solution using an indicator and is expressed in terms of lactic acid. The aim of the test is to assess suitability of milk for heat processing.

9.3.1.1 Requirements

Pipette, porcelaine dish, glass stirring rod, burette (50 x 0.1 ml), Sodium hydroxide solution (N/10), phenolphthalein solution (0.5%)

9.3.1.2 Procedure

Transfer 10 ml of milk into a white porcelain dish, with the help of pipette add 1.0 ml of 0.5% phenolphthalein solution as indicator. Titrate the contents with N/10 NaOH solution. Observe occurrence of pink color as end point for the titration. Note the titre value. Calculate percent acidity of the sample as lactic acid.

9.3.1.3 Calculation

$$\text{Titration acidity \% (as lactic acid)} = \frac{9 \times V_1 \times N}{V_2}$$

Where, V_1 = Volume of N/10 NaOH used

V_2 = Volume of milk sample

N = Normality of NaOH used

9.4 Determination of Fat in Milk and Milk Products

Fat is the most important and valuable constituent of milk and milk products. It also plays an important role in the pricing of milk and milk products. Estimation of fat in milk can be done in two ways:

1. Gerber method
2. Gravimetric method (using Rose-Gottlieb method)

9.4.1 Gerber method

9.4.1.1 Determination of fat in milk

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 will be set free which remains in liquid state due to heat produced by the acid. The amyl alcohol used facilitates the separation of a clear fat column. On centrifugation, fat being lighter will be separated on top of the solution.

a) Requirements

Gerber centrifuge, Gerber butyrometers for milk (0-10% scale with 0.1 per cent mark). Hot water bath maintained at $65^{\circ}\text{C} \pm 2^{\circ}\text{C}$. 10 ml automatic measure for acid, 1 ml automatic measure for amyl alcohol, 10.75 ml milk pipette, butyrometer stoppers, butyrometer stand, key for stoppers, Gerber sulphuric acid density 1.807 to 1.812 g/ml at 27°C corresponding with a concentration of sulphuric acid from 90 to 91% by weight. Iso- amyl alcohol 95% of clear, colorless liquid and shall distil between 130°C to 132°C having density 0.803 to 0.805 g/ml at 27°C .

b) Procedure

Take 10 ml of Gerber sulphuric acid from automatic measure into the butyrometer. 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 a slanting manner and resting the tip end on the mouth of the butyrometer. With the help of automatic pipette add 1 ml iso - amyl alcohol to the above butyrometer. Insert the stopper with the help of key and tight the stopper and mix the content by shaking the butyrometer at a 45 degree angle until all the curd has been dissolved. Keep the butyrometer in the water bath at $65^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 5 minutes. Place the butyrometer in the centrifuge and balance the machine. Centrifuge for 5 minutes, (1000-1200 rpm). After centrifuging, temper the butyrometer in the water bath at $65^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 5 minutes. With the help of key, adjust the fat column within the scale on butyrometer and take the reading and record the fat percentage. Care must be taken not to wet the neck of the butyrometer while adding Gerber sulphuric acid, milk and amyl alcohol. The test must be repeated if particles of curd are observed.

9.4.1.2 Determination of fat in cream

a) Apparatus

As in the case of milk except cream butyrometer (0- 70%) and a physical balance

b) Reagents

Fifty per cent Gerber sulphuric acid prepared by mixing equal volumes of sulphuric acid and distilled water at the time of experiment. Iso - amyl alcohol as in the case of milk.

c) Principle

Same as milk

d) Procedure

Stir the sample by carefully without causing frothing or churning. If the cream is very thick warm to 30°C-40°C to facilitate mixing. Immediately before weighing mix the sample. Keep the cream butyrometer with a small funnel at the mouth in a convenient conical flask and weight it. Then weigh accurately 5.00 ± 0.01 g of the cream into it. Add small quantities of the freshly prepared acid to the funnel to ensure complete washing down of the cream to the butyrometer. Add About 18-20 ml of the dilute acid to the butyrometer leaving sufficient space for the addition of amyl alcohol. Add one ml of amyl alcohol and proceed as in case of milk.

9.4.1.3 Determination of fat in Dahi

a) Apparatus

Same as given under milk

b) Reagents

Same as for milk

c) Principle

It is same as for milk. In addition, ammonia is added to liquefy the curd particles and to make the sample homogeneous.

d) Procedure

Weigh 100 g of the well mixed dahi sample in beaker. Add 5 ml of strong ammonia to the weighed sample and shake well to make it homogenous. Take the above prepared

sample and proceed as in the case of milk. Multiply the result obtained by the dilution factor (in this case 105/100) and add the same to the obtained result to get the actual result.

9.4.1.4 Determination of fat in cheese

a) Apparatus

Gerber centrifuge; Cheese butyrometer (0- 40%), tilt measure of 10 ml and 1 ml capacity



Fig. 9.1 Tilt measure

b) Reagents

Gerber sulphuric acid (sp. gr. 1.820 at 15.6°C); iso-amyl alcohol

c) Principle

Same as in milk

d) Procedure

Weigh accurately about 3 ± 0.01 g of cheese sample into the cheese butyrometer. Add 10 ml of warm distilled water (30°C -40°C). Add 10 ml of Gerber sulphuric acid into the butyrometer. Add 1 ml of iso-amyl alcohol. Close the butyrometer with the stopper and shake well till all the contents are well mixed. Place the butyrometer in a water bath at $65^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for tempering. Shake periodically until the solution of cheese is complete. Centrifuge at 1200 rpm for 5 minutes. Read the percentage of fat by adjusting the fat column within the scale of the butyrometer.

9.4.1.5 Determination of fat in butter

a) Apparatus

As in the case of milk except butter butyrometer (0- 90%) and a physical balance

b) Reagents

Fifty per cent Gerber sulphuric acid prepared by mixing equal volumes of sulphuric acid and distilled water at the time of experiment. Add iso-amyl alcohol as in the case of milk.

c) Principle

Same as milk

d) Procedure

Weigh accurately about 5 ± 0.01 g of butter sample in 25 ml beaker and mix it with small portion of 1:1 sulphuric acid. Transfer the contents into the butter butyrometer. Dilute sulphuric acid with distill water and add 15 to 20 ml in butyrometer. Add 1 ml of isoamyl alcohol. Rest of the method is same as in case of milk.

9.4.2 Rose-Gottlieb method

This method of fat estimation is used as a reference method. The method is a gravimetric in nature unlike Gerber method which is a volumetric method and is followed for routine purposes.

9.4.2.1 Determination of fat in milk and milk product

a) Apparatus

Fat extraction apparatus (Rose-Gottlieb tube), Oven at $100 \pm 2^\circ\text{C}$, Water bath boiling.

b) Reagents

Concentrated ammonia solution, concentrated HCl, Ethyl alcohol, Diethyl ether (solvent ether), Petroleum ether (40-60°C).

c) Principle

For quantitative separation of fat from milk and milk products, it is necessary to break up the protective film surrounding the fat globule by using suitable agents (ammonia

in case of liquid milk, and concentrated HCl in case of concentrated /dried milk products). Ammonia/Concentrated HCl brings about the break up of the protective layer. Ethyl alcohol facilitates the passage of fat from the aqueous phase to the solvents. The fat after extraction gets dissolved in the mixture of solvents (diethyl ether and pet. ether)The fat dissolved in the solvent mixture is collected in a tared conical flask containing 2-3 glass beads and then the solvents are evaporated on the water-bath and the finally dried in the oven. The weight of the dried fat in the flask is taken and percentage in the product is calculated.

d) Procedure

Weigh accurately a known quantity of well mixed sample (for example, 10 gm of liquid milk or 1 gm of concentrated/dried milk product) into the fat extraction (Rose-Gottlieb) tube. Add 1 ml conc. ammonia in case of liquid milk or 10 ml of concentrated HCl in case of concentrated/dried milk products and mix well. In case of concentrated dried milk products, heating is done on a water bath till casein has dissolved, then contents are cooled. Add 10 ml of ethyl alcohol and again mix well. Add 25 ml of diethyl ether (solvent ether) and mix properly. Then add 25 ml of petroleum ether (40-60°C) and again mix properly. Allow the extraction tube to stand till the two layers are separated clearly (Approx. 30 minutes). Siphon off the ether solution i.e. upper layer into the tared conical flask containing 2-3 glass beads. Repeat the process of extraction at least twice using 15 ml. of solvent ether and 15 ml of petroleum ether, and similarly pour these two extracts into the same tared conical flask. Evaporate carefully the solvents from the flask over a boiling water bath. Dry the residual fat in the oven at $100 \pm 2^\circ\text{C}$ for 1-2 hours. Cool the flask to room temperature in a desiccator and weigh it. Repeat the process of heating, cooling and weighing till you get a constant weight.

e) Observations

Weight of sample taken = W gm

Weight of empty tared flask with glassbeads = W_1 gm

Weight of empty tared flask with glassbeads + fat after drying = W_2 gm

Weight of fat in the flask = $W_2 - W_1$ gm = X gm.

f) Calculations

Percent fat in the milk or milk product = $X/W \times 100$

Precautions: Solvents like Diethyl ether, Petroleum ether and ammonia should be stored at low temperature before opening the bottles. There should not be any naked

flame near the place of experiment, since solvents used in this experiment are highly inflammable.

9.5 Determination of Solids-Not-Fat Content (SNF test) in Milk

9.5.1 Solids-not-fat content (SNF test) by lactometer

Lactometers are used for rapid determination of specific gravity of liquids. The method is based on the law of floatation, which states that when a solid is immersed in a liquid it is subjected to upward thrust equal to the weight of liquid displaced by it and acting vertically upwards. Lactometers are variable immersion type hydrometers and calibrated before hand with liquid of known specific gravity.

a) Requirements

Lactometer calibrated at 27°C (BIS), lactometer jar, thermometer.

b) Procedure

Adjust the temperature of milk sample to measuring temperature prescribed for lactometer (27°C). Mix the sample well to avoid incorporation of air or foam formation. Pour sufficient milk into the glass or steel cylinder to allow free floating of lactometer. Place the lactometer in the milk and allow it to float till it stops and assumes a constant level. Record the lactometer reading and temperature of milk at the same time. Take another reading by flapping the top of the lactometer stem and when it again assumes constant level. Take average of the two readings. Get corrected lactometer reading (CLR) from the standard table for corresponding temperature. Calculate solids-not-fat (SNF) content using the given formulas.

c) Calculations

$$\text{Percent SNF} = \text{CLR}/4 + 0.25 \text{ F} + 0.44$$

Where; F= Fat percentage in milk sample.

Correction factor for BIS lactometer

TEMPERATURE	FAT PERCENT OF SAMPLE				
	0	2	4	6	8
(1)	(2)	(3)	(4)	(5)	(6)
19·0	-2·2	-2·4	-2·6	-2·7	-2·9
19·5	-2·1	-2·3	-2·4	-2·6	-2·7
20·0	-2·0	-2·1	-2·2	-2·4	-2·5
20·5	-1·8	-2·0	-2·1	-2·2	-2·3
21·0	-1·7	-1·8	-1·9	-2·0	-2·2
21·5	-1·5	-1·7	-1·7	-1·9	-2·0
22·0	-1·4	-1·5	-1·6	-1·7	-1·8
22·5	-1·3	-1·4	-1·4	-1·5	-1·6
23·0	-1·1	-1·2	-1·3	-1·4	-1·4
23·5	-1·0	-1·1	-1·1	-1·2	-1·3
24·0	-0·8	-0·9	-1·0	-1·0	-1·1
24·5	-0·7	-0·8	-0·8	-0·9	-0·9
25·0	-0·6	-0·6	-0·6	-0·7	-0·7
25·5	-0·4	-0·5	-0·5	-0·5	-0·5
26·0	-0·3	-0·3	-0·3	-0·3	-0·4
26·5	-0·1	-0·2	-0·2	-0·2	-0·2
27·0	0	0	0	0	0
27·5	+0·1	+0·2	+0·2	+0·2	+0·2
28·0	+0·3	+0·3	+0·3	+0·3	+0·4
28·5	+0·4	+0·5	+0·5	+0·5	+0·5
29·0	+0·6	+0·6	+0·6	+0·7	+0·7
29·5	+0·7	+0·8	+0·8	+0·9	+0·9

(Continued)

TEMPERATURE	FAT PERCENT OF SAMPLE				
	0	2	4	6	8
(1)	(2)	(3)	(4)	(5)	(6)
30·0	+0·8	+0·9	+1·0	+1·0	+1·1
30·5	+1·0	+1·1	+1·1	+1·2	+1·3
31·0	+1·1	+1·2	+1·3	+1·4	+1·4
31·5	+1·3	+1·4	+1·4	+1·5	+1·6
32·0	+1·4	+1·5	+1·6	+1·7	+1·8
32·5	+1·5	+1·7	+1·7	+1·9	+2·0
33·0	+1·7	+1·8	+1·9	+2·0	+2·2
33·5	+1·8	+2·0	+2·1	+2·2	+2·3
34·0	+2·0	+2·1	+2·2	+2·4	+2·5
34·5	+2·1	+2·3	+2·4	+2·6	+2·7
35·0	+2·2	+2·4	+2·6	+2·7	+2·9

9.5.2 Total solid content of milk by gravimetric method

Whole milk contains about 84 –87% water and remaining 13-16% are solids comprising of fat, protein, carbohydrate and minerals. By evaporating water from the milk under controlled conditions the total solid content can be determined accurately.

a) Requirements

Aluminium moisture dish, boiling water bath/hot plate, weighing balance, hot air oven, desiccators, tong.

b) Procedure

Weigh accurately a clean and dry empty dish (A). Transfer 5.0 ml of sample into the dish and note the weight (B). Place the dish on boiling water bath or hot plate for 20-30 minutes. Allow the water to evaporate. Place the dish in hot air oven at $100 \pm 1^\circ\text{C}$ for 3 hours. Transfer the dish to a desiccator and allow cooling for 30 minutes. Weigh

the contents and note the weight (C). Repeat heating and cooling until difference in two successive weights do not exceed 0.5 mg.

c) Calculations

Weight of sample (X) = Reading B – Reading A

Weight of solids in milk (Y) = Reading C – Reading A

$$\text{Percent total solids} = \frac{(C - A)}{B - A} \times 100$$

9.6 Determination of Lactose Contents in Milk and Milk Products

The lactose in milk and milk products may be quantified by methods based on one of the five principles namely polarimetry, oxidation-reduction titration (Lane- Eynon method), colorimetry, chromatography and enzymatically. Polarimetric method is simple, accurate and rapid for the estimation of lactose in milk and milk products. However, Lane- Eynon method is most commonly used and widely accepted method.

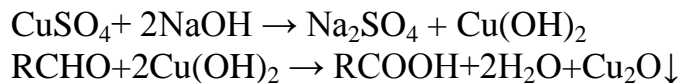
9.6.1 Lane-Eynon method

9.6.1.1 Principle

Reducing sugars, which are more common, are able to function as reducing agents because of free aldehydic/ketonic groups present in the molecule. The reducing properties of these sugars are usually observed by their ability to reduce metal ions notably copper, iron and silver in alkaline solution. Those very properties of sugars have been used in this method. The reducing sugar solution reduces an alkaline cupric-salt solution during boiling and converts into the red cuprous-oxide. From the reduced amount of copper salt, the quantity of reducing sugar is estimated. Fehling solution is a mixture of Fehling A ($\text{CuSO}_4 \cdot \text{H}_2\text{O}$) and Fehling B (alkaline sodium potassium tartarate). When copper sulphate is made alkaline, it gives the blue colored precipitates of $\text{Cu}(\text{OH})_2$. But the presence of sodium potassium tartarate forms a soluble blue colored complex of copper compound behaves as if it is alkaline $\text{Cu}(\text{OH})_2$ solution. In general, when NaOH is added CuSO_4 , a blue precipitate of $\text{Cu}(\text{OH})_2$ is obtained (Fehling A). These precipitates are made to dissolve in Rochelle salt solution (Fehling B) and a blue color solution is obtained, which is known as Fehling solution. The Fehling solution when heated gives rise to cupric oxide (CuO) which in

turn reacts with reducing sugar and gets reduced to cuprous oxide (Cu_2O) brick red precipitate and resulting into the oxidation of sugars to corresponding acids.

9.6.1.2 Reactions



9.6.1.3 Reagents

a) Fehling solution A

Weigh 34.639 gm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and dissolve in distilled water. Add 0.5 ml of Concentrated H_2SO_4 and make the volume to 500 ml.

b) Fehling solution B

Weigh 173 gm Rochelle salt and 50 gm NaOH and dissolve in distilled water and make the volume to 500 ml.

c) Zinc acetate solution (2N)

Dissolve 21.9 gm of crystallized zinc acetate $[\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot \text{H}_2\text{O}]$ in distilled water add 3 ml of acetic acid and make up the volume to 100 ml.

d) Potassium Ferrocyanide (1N)

Dissolve 10.6 gm Potassium Ferrocyanide, $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$ in distilled water and make upto 100 ml.

e) Acetic acid solution

Equivalent to dilute NH_3 strength.

f) Dil. aqueous ammonia solution (10%)

10 ml Conc. NH_3 , diluted to 100 ml.

g) Aqueous methylene blue indicator 0.2%

Dissolve 0.2 g of methylene blue in distilled water and make the volume to 100 ml.

h) Standard lactose solution (5%)

Dissolve 5 gm lactose in distilled water and make the volume to 100 ml.

9.6.1.4 Procedure

a) Standardization of Fehling solution

Pipette out 5 ml of Fehling solution A and 5 ml of Fehling solution B in a 100-150 ml conical flask and mix. Take the standard lactose solution in a burette. Warm the contents in a conical flask over the burner and add a little less than expected amount of sugar solution and allow it to boil. Add a few drops of methylene blue. Now add drop wise the sugar solution from the burette till the blue color disappears and brick red color appears. By this method, determine the actual amount of standard solution utilized against 5 ml of aqueous solution.

b) Sample preparation

Weigh accurately 40 gm of sample in a 100 ml beaker, to that add 50 ml of hot water (30-90°C). Mix well. Transfer the contents to a 250 ml volumetric flask and rinse the beaker with hot water to make the volume to about 120-150 ml. Add 5ml of 10% dil. NH₃ solution, mix well for 10-15min. Add 5 ml of 10% acetic acid to neutralize ammonia (equivalent amount of glacial acetic acid). Add 12.5 ml of zinc acetate solution and 12.5 ml of Potassium Ferrocyanide solution to precipitate the proteins and mix well. Make up the volume to 250ml with distilled water. Filter through Whatman No.1. Discard some amount of filtrate and the rest is used for the titration.

c) Estimation of reducing sugar

Take 25 ml of this sample solution (filtrate), dilute it to 100 ml and titrate against 10 ml Fehling solution (5 ml Fehling A + 5 ml Fehling B).

d) Calculation

10 ml Fehling (A+B) solution = V_1 ml of standard lactose sugar solution of concentrated C_1 mg/l

10 ml Fehling (A+B) solution = V_2 ml of sample solution of Concentration C_2

$$V_1 C_1 = V_2 C_2$$

Therefore,

$$C_2 = \frac{V_1 C_1}{V_2} \text{ mg/ml}$$

$$C_2(\text{lactose}) = \frac{V_1 C_1 \times 250 \times 100}{V_2 \times 1000 \times 40 \times 25} \times 100\%$$

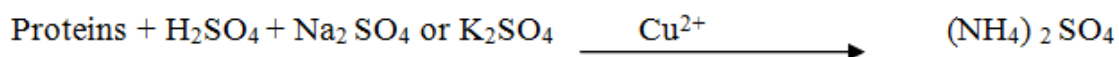
9.7 Determination of Protein Contents in Milk and Milk Products by IDF and BIS Methods

9.7.1 Principle

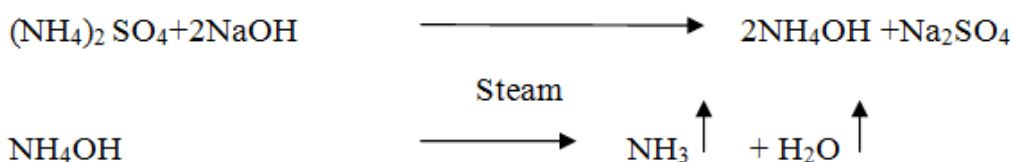
The test portion is digested using a block-digestion or equivalent apparatus with a mixture of concentrated sulfuric acid and potassium sulfate, using copper (II) sulfate as a catalyst to convert organic nitrogen to ammonium sulfate. Addition of excess sodium hydroxide to the cooled digest liberates ammonia. The liberated ammonia is distilled, using either a manual or semi-automatic steam distillation and collected into an excess of boric acid solution followed by titration with hydrochloric acid solution. The nitrogen content is calculated from the volume of HCl used by ammonia in the titration and multiplied by 6.38 to get the corresponding crude protein content.

9.7.2 Chemistry of nitrogen determination

A. Solubilization stage



B. Release of ammonia and steam distillation



C. Collection of ammonia and back titration



9.7.3 Conversion factor

Proteins in general vary in nitrogen content from 14 to 19% and thus a single universal conversion factor cannot be used. An average factor of 6.38 (corresponding to 15.65% N) is commonly used for milk proteins to convert nitrogen to protein.

9.7.4 Sample size

The protein content of different types of milk and milk products varies so for determination of total protein content by Kjeldahl method the size of sample varies such that the test portion sample should contain 0.2 to 0.4 g of protein.

9.7.5 Apparatus

Water bath; Analytical balance; Digestion block; Digestion tube, 250 ml capacity; Exhaust manifold; Aspirator; Automatic pipettes (dispensers); Graduated measuring cylinder 50 ml capacity; Distillation unit; Conical flask 250 ml capacity; Burette 25 ml capacity.

9.7.6 Reagents

- Kjeldahl catalyst mixture (Digestion mixture): It consists of 3.5 g potassium sulfate and 0.105 g copper sulfate.
- Sulfuric acid: with a mass fraction of at least 98% nitrogen free.
- Sodium hydroxide solution: Nitrogen free, containing approximately 40 g sodium hydroxide per 100 ml.
- Boric acid solution: Dissolve 40 g of boric acid in 1 litre of hot water in a 1000 ml one-mark volumetric flask. Allow the contents to cool to 20°C and adjust the mark with water.
- Indicator solution: Dissolve 0.25 g of methylene blue and 0.375 g of methyl red in 300 ml of 95 per cent ethanol.
- Hydrochloric acid 0.1N
- Tryptophan or lysine hydrochloride, minimum assay 99% (mass fraction).
- Sucrose, with nitrogen content not more than 0.002%.

9.7.7 Procedure

9.7.7.1 Preparation of test sample

Warm the test sample to between 38°C to 40°C in the water bath. Cool the sample to room temperature, while gently mixing the test sample immediately prior to weighing the test portion.

9.7.7.2 Test portion and pre-treatment

To a clean and dry digestion tube, add 5 g of digestion mixture. Weigh 2 g of test sample to the nearest 0.1 mg into the tube. Carefully add 10 ml of sulfuric acid along the sides of the digestion tube. Gently mix the contents of the tube and then leave to stand for 10 min.

9.7.7.3 Digestion



Fig. 9.2 Digestion Block

Set the digestion block at a low initial temperature so as to control foaming (approximately 180°C). Transfer the tubes to the digestion block and place the exhaust manifold which is itself connected to a water jet pump in the top of the tube. The suction rate of the water jet pump should be just sufficient to remove fumes. Digest the sample until white fumes develop. Then increase the temperature of digestion block to between 410°C and 430°C and continue digestion of the sample until the digest is clear. After the digest clears (clear with light blue-green color), continue digestion at between 410°C and 430°C for at least 1 h. During this time the sulfuric acid should be boiling. If visible boiling of the clear liquid is not apparent as bubbles forming at the surface of the hot liquid around the perimeter of the tube, then the temperature of the block may be too low. The total digestion time will be between 1.75 h and 2.5 h. At the end of the digestion, the digest shall be clear and free from undigested material. Remove the tube from the block with the exhaust manifold in place. Allow the digest to cool to room temperature over a period of approximately 30 min. The cooled digest should be liquid with a few small crystals at the bottom of the tube. Excessive crystallization indicates too little residual sulfuric acid at end of the digestion and may cause a decrease in protein estimation results. To reduce acid loss during digestion, reduce aspiration rate. After the digest has cooled to room temperature in approx. 30 min, remove the exhaust manifold and carefully add 50 ml of water to each tube. Swirl to mix while ensuring that any separated out crystals are dissolved. Allow the contents of the tube to cool to room temperature again.

9.7.7.4 Distillation



Fig. 9.3 Distillation unit

Transfer the digestion tube to the distillation unit and place a conical flask containing 50 ml of boric acid solution under the outlet of condenser in such a way that the delivery tube is below the surface of the excess boric acid solution and run the programme for automatic distillation. Adjust the distillation unit to dispense 60 ml of sodium hydroxide solution and distill off the ammonia liberated by the addition of sodium hydroxide solution. Following the manufacture's instructions, operate the distillation unit in such a way as to steam distil the ammonia liberated by addition of sodium hydroxide solution, collecting the distillate in the boric acid solution containing mixed indicator. Continue with the distillation process until at least 150 ml of distillate is collected. Remove the conical flask from the distillation unit and completely drain the distillation tip. Rinse the inside and outside of the tip with water, collecting the rinsing in the conical flask. Always rinse the tip with water between samples.

9.7.7.5 Titration

Titrate the contents of the conical flask with the 0.1N hydrochloric acid using a burette and read out the amount of titrant used. The end point is reached at the first appearance of violet color in the contents.

9.7.7.6 Blank test

Carry out a blank test following the procedure described above taking 5 ml of water and about 0.85 g of sucrose instead of test portion.

9.7.7.7 Recovery tests

The accuracy of the procedure should be checked regularly by means of recovery tests as given below:

a) Check that digestion and distillation procedures are efficient by using a test portion

of 0.06 g of lysine hydrochloride or 0.08 g of tryptophan weighed to the nearest 0.1 mg.

b) Determine the nitrogen content according to the procedure described above (9.7.7). The expected nitrogen content is 15.33% in lysine and 13.72% in tryptophan (the nitrogen recovery should be (98.5% to 101%).

c) Prepare a solution of ammonium sulphate of concentration 0.05 mol/L exactly. Pipette a 10 ml aliquot of the ammonium sulphate solution into the digestion tube and add 50 ml of water. Determine the nitrogen content of the solution according to the procedure described in 9.7.7 (nitrogen recovery should be 99% to 101%).

9.7.7.8 Calculation

Calculate the nitrogen content, W_n , by using the following equation:

$$W_n = \frac{1.4 (V_s - V_b) N}{m}$$

Where,

W_n = is the nitrogen content of the sample, expressed as percentage by mass
 V_s = is the numerical value of hydrochloric acid solution used in determination in millilitres, expressed to the nearest 0.05 ml

V_b = is the numerical value of the volume of hydrochloric acid solution used in the blank test in millilitres, expressed to the nearest 0.05 ml

N = is the numerical value of the exact normality of the hydrochloric acid solution expressed to four decimal places.

m = is the numerical value of the mass of the test portion in grams, expressed to the nearest 1 mg,

Calculate the crude protein content W_p using the following equation:

$$W_p = W_n \times 6.38$$

where, W_p = is the crude protein content, expressed as a percentage by mass.
 W_n = is the nitrogen content of the sample, expressed as a percentage by mass to four decimal places.

= is the generally accepted multiplication factor to express the nitrogen content as crude protein content

9.7.8 BIS method

9.7.8.1 Apparatus

i) Digestion flask – Kjeldahl flask

ii) Distillation apparatus – Micro Kjeldahl distillation

The micro Kjeldahl apparatus for steam distillation is shown above. Steam is produced by boiling water in flask A, and this is bubbled through the solution in flask C. The distillate passes through the condenser D and can be collected at the end of the condenser. A trap B is provided between flasks A and C in such a way, that when A is cooled, the contents in C are sucked out into flask B which can then be discarded. There is also a funnel arrangement F provided to add samples directly to flask C.

9.7.8.2 Reagents

- a) Concentrated sulfuric acid – approx. 98% by weight and nitrogen free ($\rho=1.84 \text{ g/cm}^3$)
- b) Copper sulphate
- c) Potassium sulphate or anhydrous sodium sulphate (nitrogen free)
- d) Sodium hydroxide solution – 50% by wt.
- e) Boric acid solution – saturated
- f) Indicator solution – Mix equal volumes of a saturated solution of methyl red in ethanol (95% by vol.) and a 0.1% solution of methylene blue in ethanol (95% by vol.)
- g) Standard hydrochloric acid – 0.02N

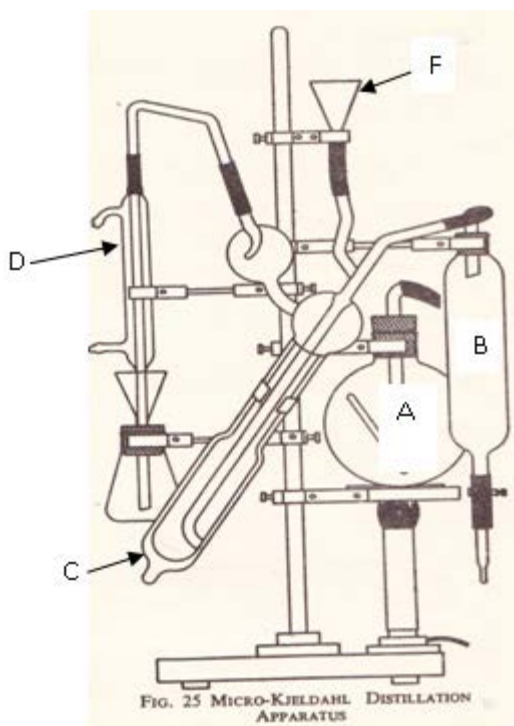


Fig. 9.4 Micro Kjeldahl distillation apparatus

9.7.8.3 Preparation of test sample

Warm the test sample of milk between 38°C to 40°C in the water bath. Cool it to room temperature while gently mixing the test sample immediately prior to weighing the test portion.

9.7.8.4 Procedure

a) Digestion of sample

Transfer accurately weighed (approx.) 10 g sample of milk to a Kjeldahl flask. Add 10 g of potassium sulphate and 0.2 g copper sulphate. Add 25 ml of concentrated sulfuric acid, along the neck of the flask in such a way as to wash down any milk drops sticking to the side of the flask. Gently rotate the flask so that the whole of the contents are well mixed. Place the flask on a flame so that the neck is inclined at an angle of 45° to the horizontal and the bulb rests in the hole of an asbestos sheet so that the flame does not touch the flask above the level of the liquid. Heat initially to gentle boiling and when frothing has ceased, boil the contents of the flask briskly until clear and free from yellowish color and for a further period of one hour. Allow the liquid to cool and wash down the sides with a fine jet of distilled water. Continue heating the contents of the flask for a further period of one hour. Allow the liquid to cool to room temperature and make up to volume in a 100 ml volumetric flask.

b) Distillation

Pipette out 10 ml aliquot of the solution into the flask 'C' through the funnel of micro Kjeldahl distillation apparatus. Then add 8 ml of sodium hydroxide solution through funnel. Keep a flask containing 10ml of the boric acid solution containing 2-3 drops of the indicator, at the delivery end of the condenser in such a way that the tip is just beneath the surface of the liquid. Now heat the flask 'A' filled with water to produce steam. This steam is passed through the contents of flask 'C'. The ammonia evolved there by the alkaline treatment of digested sample is carried along with steam through the condenser outlet and is absorbed in boric acid solution. Continue passing steam for 10 minutes and collect about 50 ml of distillate, then remove the receiver flask after rinsing out the tip of the condenser. Stop heating flask 'A'. On cooling, this will create a back suction so that the contents in the flask 'C' will be sucked into the trap 'B'. Add about 10 ml of water through the funnel quickly, so that it will also be sucked into flask 'B', while rinsing flask 'C'. The apparatus is now ready for distillation of next sample. Titrate the contents in the receiver flask against the standard hydrochloric acid till the color changes from green to violet color. Note the volume of acid used. Carry out a blank determination by taking 0.5 g of sucrose in place of milk, and by using the same quantities of reagents and the same conditions of test.

c) Calculations

Crude protein is calculated by multiplying nitrogen content by the factor 6.38.

$$\text{Crude protein (percent by wt.)} = \frac{6.38 \times 1.4 (V_2 - V_1) N \times DF (100/10)}{W}$$

Where,

V_2 = Number of ml of hydrochloric acid standard volumetric solution used in distillation

V_1 = Number of ml of hydrochloric acid standard volumetric solution used in the blank test

N = Normality of hydrochloric acid standard volumetric solution

W = Weight in g of the sample taken for analysis

DF = dilution factor

9.8 Total ash content

9.8.1 Principle

Milk contains soluble salts like sodium, potassium, calcium, phosphorus, citrates, sulphates, chlorides, carbonates, magnesium etc. Heating milk at higher temperatures decomposes organic matter and the soluble inorganic salts are left in the form of ash.

9.8.2 Requirements

Single pan balance, muffle furnace, desiccator, silica crucible, tong, hot plate.

9.8.3 Procedure

Accurately weigh 10 g of milk sample in to the silica crucible. Evaporate the sample to dryness on a hot plate. Place the crucible in a pre-heated muffle furnace and heat the contents at 550°C until ash is free from carbon. Cool the crucible by placing in desiccator. Weight the crucible containing the ash. Continue heating, cooling and weighing until the difference in weight is not more than 0.1 gm.

9.8.4 Calculations

weight of empty crucible = W g

weight of crucible with milk = W_1 g

weight of crucible after drying = W_2 g

weight of milk sample = $W_1 - W$ g (A)

weight of ash = $W_2 - W$ g (B)

$$\text{Percent ash by weight} = \frac{B}{A \times 100}$$