UNIT 7 CHEMICAL ANALYSIS OF MILK AND MILK PRODUCTS

Structure

7.0 OBJECTIVES

After reading this unit we should be able to:

- Perform different platform tests to accept or reject the milk
- Determine different milk constituents
- Analyze different milk products for their quality
- Analyze the water used in dairy plant for different attributes.

7.1 INTRODUCTION

Analysis is done to know the composition of a product. Analysis determines not only the quality of the product but also the quantity of ingredients required in manufacturing. Payment is based on certain important parameters of the product. In case of milk it is Fat and SNF percent. In the industry Fat by Gerber Method and SNF by using Lactometer are estimated. In some dairies Electronic Milk Tester is used for determination of Fat in milk. Very few dairies use Milkoscans for the determination Fat, Protein, Lactose and SNF. To determine the quality of a product, tests for acidity, adulterants, preservatives etc have to be carried out. Knowledge of methods, good laboratory practices and good testing skills are required in the analysis of milk and milk products.

7.2 TEST METHODS

Title	Sub-Titles
Testing of Milk	**Platform Tests:**
	Clot on Boiling (COB) Test
	Alcohol Test (To determine heat stability of milk)
	Determination of Titratable Acidity
	Determination of Preservative and Adulterants

Hydrogen Peroxide

Hypochlorites

Formaldehyde (Honnies Test)

Boric Acid and Borates

Maltodextrins

Urea

Neutralizers

Starch

Sugar

Salt

Mineral Oil

Other Tests:

Determination of milk fat (Gerber Method)

Testing Fat in Homogenized milk

Microscopic observation of fat globules size

Determination of SNF (Volumetric Method)

Determination of Total Solids

Phosphatase Test

Determination of Ash content in milk

Determination of Protein in milk

Testing of Milk Powder

Moisture Content by IMA

Moisture Content by drying method

Titrable Acidity

Rosalic Acid Test

Scorched Particles

Ash Content

Insolubility Index

Fat Percent (WMP)

Fat percent (SMP)

Bulk Density

Testing of Butter

Determination of Moisture

Determination of Curd

Determination of Fat

Titrable Acidity

Analysis of Salt in Table Butter

Testing of Ice Cream	Determination of Fat
	Determination of Protein
	Titratable Acidity
	Determination of Total Solids
	Phosphtase Test
	Titratable Acidity (Candy Mix)
	Determination of Total Solids (Candy Mix)
Testing of Paneer	
	Determination of Moisture
	Determination of Fat
	Determination of Acidity
Testing of Ghee	
	Determination of Moisture
	Free Fatty Acids Percent as Oleic Acid
	Butyro Refractometer Reading
	RM Test
Testing of Flavoured Milk (UHT Milk)	
	Determination of Fat
	Determination of Total Solids
	Determination of Acidity
Testing of Sterilized Cream	
	Determination of Fat
	Determination of Acidity
Testing of Lassi	Determination of Fat
	Determination of Total Solids
	Determination of Acidity
Testing of Curd	Determination of Fat
	Determination of Acidity
Testing of Water	Hardness
	PH
	Sulphite Ions (For Boiler Water)
	Phosphate Ions (For boiler water)
	Residual Chlorine in Water by Tolidine Method
	Residual Chlorine in Water by Chlorotex Method
Preparation	Chemical Solutions/Reagents

PLATFORM TESTS

Clot on Boiling Test :

Apparatus:

Test Tubes, Spirit Lamp

Principle:

To detect in a rapid manner the presence of extent of developed acidity, which might render the milk unsuitable for processing and distribution.

Procedure:

Conduct COB test by taking about 5 ml of milk in a test tube, boil on the flame of a spirit lamp.

Observation:

Formation of clots in the test tube indicates COB positive milk and is unacceptable.

Alcohol Test (To determine heat stability of milk) :

Apparatus:

Test Tubes

Reagents:

80% ethyl alcohol; 60% ethyl alcohol

Principle:

This test is conducted to know the heat stability of milk. It is useful in the manufacture of condensed milk, UHT, Dried and Pasteurized milk. 80% ethyl alcohol is recommended for selection of milk to process under UHT system. For selection of milk to pasteurize, 60% ethyl alcohol is recommended. One of the factors causing heat instability of milk is disturbance in mineral balance.

Procedure:

Take 5 ml of milk in a test tube. Add the desired percent of ethyl alcohol in equal quantity. Shake the contents. Observe for clots.

Observation:

Absence of clots indicate that the milk is suitable for respective heat treatment.

Determination of Titratable Acidity

Apparatus:

Burette, Conical flask (100 ml capacity), Stirring Rods, Pipette (10 ml) & Tilt Measure (1 ml) for indicator.

Reagents:

N/10 NaOH, Phenolphthalein indicator.

Principle:

When freshly drawn, milk contain very low acidity acid contributed by its constituents, namely, carbondioxide, citric acid, albumin, casein and minerals. Later during storage due to bacterial action on lactose acidity increases. Since alkali neutralizes acid the acidity of milk is estimated through titration against standard alkali using phenolphthalein as indicator. Normal acidity of milk range from 0.14% to 0.16%. Lower range (less than 0.12%) indicate neutralization and higher range (more than 0.16%) indicate development of acidity due to bacterial action or mastitis milk.

Procedure:

Take 10 ml milk in 100 ml conical flask, add 10 ml distilled water. Add 1ml phenolphthalein indicator and titrate against N/10 NaOH till a faint pink colour appears to determine the percent lactic acid in milk.(The pink color has to match with rosaniline acetate bench solution.)

Calculation:

Calculate the acidity % as volume of NaOH used X 0.09.

Check Your Progress - 1

1. Why COB test is conducted?

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2. What is the principle of Alcohol Test?

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3. Where this test is most useful?

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4. What substances are responsible for the Acidity in milk?

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5. What is the significance of developed acidity in milk?

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Detection of Preservatives and Adulterants

Note: For detection use Kits developed by NDDB. These Kits contain prepared solutions and glassware that can be used at the site of testing. They are suitable for detection of urea, ammonia fertilizers, nitrate pond water, starch and cereal flours, sugar/sucrose, glucose, salt, neutralizers, hydrogen peroxide and formalin. The Kits are easy to carry and cost effective. However for the benefit of students some test methods are given below:

Hydrogen Peroxide

Apparatus:

Test Tubes

Reagents:

2% aqueous solution of paraphenyl diamine hydrochloride (fresh solution).

Principle:

It is a preservative. It is not permitted under the law to be added to milk or milk products for consumption.

Procedure:

Method-1

Take 10 ml of milk in a test tube. Add 2 drops of PPDH. Mix thoroughly and observe.

Observation:

Blue colour indicates the presence of hydrogen Peroxide.

Hypochlorites

Apparatus:

Test tubes, water bath.

Reagents:

7% potassium iodide solution, 50% hydrochloric acid, 1% starch solution.

Principle:

Hypochlorites act as preservatives and sterilizing agent in milk. They are not permitted under the law to be added to milk or milk products.

Procedure:

- Take 5 ml of milk and add 1.5 ml potassium iodide solution (7%) and mix. A pale yellow or yellowish brown colour indicates the presence of hypochlorites.
- If no colour appears add 4 ml of hydrochloric acid to the above mix. Mix thoroughly and keep in water bath at 85^{O}C for 10 minutes. Cool by immersing in cold water and add 1 ml of starch (1%) allowing it to flow down below the curd.

Observation:

A blue to purplish red colour indicates the presence the presence of Hypochlorites.

Formaldehyde (Hehner Test)

Apparatus:

Test Tubes.

Reagents:

Ferric Chloride (1%), Conc. Sulphuric Acid.

Principle:

It acts as preservative. It is permitted only in samples to be used in chemical analysis. Not permitted to be used in milk or milk products used for consumption.

Procedure:

Take 10 ml of milk in a test tube. Add 0.5 ml of Ferric Chloride (1%). Mix. Add carefully 5 ml Conc. Sulphuric Acid down the side of the test tube in such a way that it forms a separate layer at the bottom without mixing with the milk.

Observation:

Characteristic violet colour ring at the junction indicates presence of Formaldehyde.

Boric Acid and Borates

Apparatus:

Test Tubes

Reagents:

Conc. HCI; Turmeric Paper; NH_4OH (28%)

Principle:

It acts as preservative. Not permitted by the law to be added in milk and milk products.

Procedure:

- Take about 5 ml of milk in a test tube.
- Add about 1 ml of conc. HCI and mix well.
- Dip a strip of turmeric paper in the acidified milk.
- Dry the paper immediately and note the change in colour.

Observation:

Turmeric paper turning into red indicates the presence of Boric Acid and Borates. Further confirmation is done by adding a drop of ammonium hydroxide. Colour changes to dark green indicate the presence of Boric Acid.

Maltodextrins

Apparatus:

Test Tubes, beakers, pipettes, Whatman filter paper No.42, funnels

Reagents:

10% Citric acid solution

1% Iodine solution

Principle:

To increase the density of normal milk. It is an adulterant.

Procedure:

Take 20 ml of milk sample in a beaker. Boil & coagulate the milk using 10% citric acid solution. Filter through Whatman filter paper No.42 & collect the filtrate. Add 3 drops of iodine solution to 5 ml. filtrate & mix well.

Observations:

The appearance of chocolate brown colour indicates the presence of maltodextrins.

Urea

Reagents:

P-Dimethylamino Benzaldehyde (DMAB) solution: Dissolve 16 gm in 1 litre of alcohol and add 100 ml previously chilled Hcl. Stable for 1 month.

Principle:

It is added to increase the density of normal milk. It is an adulterant. Normal milk in this test gives light yellow colour urea is a natural constituent of milk. However more than 700 ppm is considered as added urea.

Procedure:

Take 2 ml milk in a test tube, add 2 ml DMAB solution and mix the contents. Appearance of **yellow colour** indicates the presence of urea making the milk unacceptable.

Rosalic Acid

Reagent:

Ethyl alcohol (95%), Rosalic Acid

Principle:

Rosalic develops a rose red colour with milk containing alklies, where as it gives only a brownish colouration with pure milk.

Procedure:

(a) To 5 ml of milk in a test tube, add 5 ml of alcohol , Add few drops of one percent (w/v) alcoholic solution of rosalic acid and mix. If neutralizer is present, a rose red colour appears whereas pure milk shows only a brownish colouration.

(b) Take 2 ml rosalic acid solution (0.05% in 60:40 alcohol and distilled water) in a test tube, add 2 ml of milk. Rose-Red colour development indicates neutralizer presence in milk.

Note: Method (b) is commonly used in dairies to know heat stability and detection of added neutralizers, in milk.

Starch

Reagent:

Iodine solution (1%)

Principle:

Iodine solution gives intense blue colour with starch due to formation of unstable complex starch-iodo compound. Added to increase the density of normal milk. (Adulterant).

Procedure:

Take 3 ml. milk in a test tube, boil and cool under tap water. Add a drop of 1% Iodine solution.

Observation:

Presence of starch is indicated by the appearance of a blue colour which disappears when the sample is boiled and re-appears on cooling.

Sugar

Reagent:

Conc. HCl, Resorcinol

Principle:

Resorcinol produces red colour with sucrose in acidic media. Sugar increases specific gravity of milk. Unless permitted specifically under law it is considered as an adulterant.

Procedure:

(a) To about 15 ml. of milk in test-tube, add one millilitre of concentrated hydrochloric acid and 0.1gm of resorcinol and mix. Place the tube in boiling water-bath for five minutes.

Observation:

In the presence of cane sugar, a red colour is produced.

(b) Take 3 ml of milk in a test tube and add 5 ml dilute HCl (1:2) containing resorcinol (0.1 gm. resorcinol dissolved in 100 ml dilute HCl). Mix well and keep the test tube in boiling water for 5 minutes.

Observation:

In the presence of cane sugar, a red colour is produced.

Salt

Reagent:

Silver nitrate solution (0.1341 %), Potassium chromate (10%)

Principle:

It is added to increase the density of normal milk (adulterant). Sometimes brine solution can enter milk by accident during chilling.

Procedure:

Take 5 ml of silver nitrate (0.1341%) solution in a test tube and add two drops of potassium chromate (10%) solution. It will give brick red colour. To this add exactly 1 ml of milk and mix.

Observation:

Appearance of yellow colour show the presence of salt.

Mineral Oil (Holde's Test)

Reagent:

Alcoholic KOH.

(Dissolve 7 gms. KOH pallets in 10 ml distilled water and make the volume to 250 ml by adding rectified spirit).

Principle:

Mineral oil is an adulterant. It is added to give a false fat% during fat testing.

Procedure:

Take 1 gm of ghee extracted from milk in a 250 ml conical flask and to this add 22 ml alcoholic KOH and keep the flask on boiling water bath or hot plate and saponify it till there is no oil droplets. To this add 25 ml of boiling distilled water and swirl the flask. **Development of turbidity indicates the presence of mineral oil** & such milk is unacceptable. No turbidity indicates absence of mineral oil.

Sample found unacceptable at any stage during platform tests renders the milk unfit for acceptance. Confirm the results by drawing second sample. Reject such milk

Check Your Progress - 2

1. Why preservatives and adulterants are added to milk?

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2. It maltrodexbin a preservative or adulterant?

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3. What is the objective in adding sugar and mineral oil to milk?

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I. Testing of Milk

i) Determination of Milk Fat :

Gerber Method:

Apparatus:

- Butyrometer 10% scale (0-10% scale with 0.1% mark)
- 10 ml automatic measure for sulphuric acid
- 10.75 ml pipette for milk
- 1 ml automatic measure for amyl alcohol
- Stoppers for butyrometer
- Gerber Centrifuge (1400 ± 70 RPM)
- Water bath (65 ± 2^{O}C)
- Butyrometer stand
- Lock Stopper Key

Reagents:

Sulphuric acid (Specific Gravity 1.807 to 1.812 gm/ml at 27^{O}C) corresponding to a concentration of 90 to 91% by mass which is normally called as Gerber Acid.

Amyl alcohol (Specific Gravity 0.810 to 0.812 gm/ml at 27^{O}C) conforming to grade 1 of IS:360:1964 of clear and colourless liquid shall distil between 130^{O}C to 132^{O}C.

Principle:

Rapid Method of estimation of Fat in the fluid milk is known as “Gerber Method”. It is based on the principle of measuring the volume of Fat released from a known volume of the milk sample in a specially devised and accurately calibrated modified form of glass cylinder called Butyrometer.

When a definite quantity of sulphuric acid and amyl alcohol are added to a definite volume of milk, the proteins will be dissolved and fat globules will be set free with the help of amyl alcohol which remains in liquid state due to heat produced by the acid. During centrifugation fat being lighter (fat density 0.90 gm/ml at 40^{O}C) gets separated and comes to the top of the solution.

Procedure:

- Transfer 10 ml of sulphuric acid into the butyrometer by means of automatic measure taking care not to wet the neck of the butyrometer with the sulphuric acid.

- Warm the sample to approximately 27^{O}C and mix thoroughly but do not shake it so vigorously as to cause churning of the fat. Allow the sample to stand for 3-4 minutes after mixing to allow air bubbles to escape, invert the sample bottle 3-4 times immediately prior to taking milk for test.

- Transfer 10.75 ml of sample into the butyrometer by using 10.75 ml milk pipette by following the below mentioned procedure.

- Add 1 ml of amyl alcohol into the butyrometer by means of automatic measure and close the neck of the butyrometer firmly with a stopper without disturbing the contents. Shake the butyrometer carefully without inverting it until the contents are thoroughly mixed, the curd is dissolved and no white particles are seen in the liquid. Then invert the butyrometer few times to mix the contents thoroughly. (It is always safe to use butyrometer stand while mixing/shaking the contents.)

- Transfer the butyrometer quickly in the waterbath at 65 ± 2^{O}C and leave it there for not less than 5 minutes.

- Take out the butyrometer out of the water bath and centrifuge at 1400 rpm for 4 minutes. Bring the centrifuge to stop gradually, transfer the butyrometers (stoppers downwards) into the water bath at 65 ± 2^{O}C and allow the butyrometer to stand for not less than 3 minutes and not more than 10 minutes and take down reading.

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

- Use lock stopper key for fixing and removing the stopper into / from the butyrometer and also for adjusting the fat reading.

Procedure for Testing Fat in Homogenized Milk

In case of homogenized milk, repeat the temperature adjustment and centrifuging before taking the reading. If the second value does not exceed the first value by more than half a smallest scale division of the butyrometer, the second value shall be recorded as the fat content of the milk.

Microscopic Observation of Fat Globule size to assess Homogenization Efficiency

Principle :

Homogenization of milk and cream is done to make fat globules into smaller and equal size of about 2 microns. It helps in the distribution of fat uniformly in the system and also delays/almost arrest the rise of fat to the top.

Apparatus:

- Microscope fitted with low, high and oil immersion objectives and 10 X eye piece.

- Microscope Slides

- Cover glass.

- Graduated cylinder 100 ml.

- Pipette 1 ml
- Test tubes 150 ml.
- Micrometer fitted in the eye-piece.

Procedure:

- Dilute the sample to be tested with distilled water so that the sample contain from 0.1 to 0.2% Fat. The following table give the approximate dilution to use:

  Milk -dilute 1 ml with 25 ml distilled water.

  20% Cream -dilute 1 ml with 150 ml distilled water.

  30% Cream- dilute 1 ml with 225 ml distilled water.

  40% Cream -dilute 1 ml with 300 ml distilled water.

  10% Ice Cream- dilute 1 ml with 75 ml distilled water.

  20% Ice Cream -dilute 1 ml with 150 ml distilled water.

- Prepare a slide and observe under oil immersion. Note the size of the fat globules through micrometer fitted in the eye-piece.
- Observations: In homogenized milk 90% of the fat globules should be within 2 micron size. No lumps should be present. No clumping formation should be there.

ii) Determination of SNF

Volumetric Method

Apparatus:

Calibrated Lactometer at 15.5^{O}C, Lactometer Jar (suitable to float the Lactometer), Calibrated Thermometer and Enamel Tray.

Principle:

The constituents of milk are broadly divided into fat and solids-not-fat.The major components of SNF are proteins and lactose. Fat is estimated more easily by Gerber method. The specific gravity of milk is measured using a lactometer. Corrections are made to the reading. Fat reading is taken using Gerber method. A formula is used to workout SNF. Some constant factor derived from gravimetric analysis is taken into calculation to get the results near to Gravimetric results. Composition of milk (Cow/ Buffalo/ Breeds/ Season/ Feeding pattern) , the constant factor, calibration of lactometer and Adulteration play a major role in determing SNF using lactometer.With all these drawbacks it is still considered an important test along with fat estimation in the Q.C. Laboratory.

Procedure:

- Warm the milk sample to 40^{O}C to 45^{O}C and maintain at this temperature for 5 minutes.

- Mix the contents by rotating and inverting the bottle, taking care to avoid the formation of air bubbles and froth.
- Cool the sample approx. near to the calibrated temperature of the Lactometer ($15.5^{O}C$).
- Invert the sample bottle two or three times, pour enough milk into the lactometer jar taking care to avoid the formation of air bubbles, so that some milk overflows when the lactometer is inserted.
- 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.
- Allow the lactometer to remain steady in the milk. Take the reading at $15.5^{O}C$ within 30 seconds. Note the reading of the lactometer corresponding to the top of the meniscus on the stem without the error of parallax.
- Determine the fat percentage as per Gerber Test Method.

Calculation of Solids not Fat:

Formula :

$$SNF\% = \frac{CLR}{4} + 0.2 + 0.29$$

When CLR = Corrected lactometer reading

F = Fat Percentage

Note: The constant factor 0.29 is an example for lactometer at 15.5 degree C. Lactometer at 84^{0} F can also be used. By performing gravimetric analysis one has to arrive at the constant factor specific to the area of operation.

iii) Determination of Total Solids

Gravimetric Method:

Apparatus:

Shallow flat bottomed dishes of aluminium alloy, nickel, stainless steel, porcelain or silica, 7 to 8 cm diameter, about 1.5 cm in height and provided with easily removable lid. Hot air oven maintained at $100^{O}C$, $\pm^{0}C$ Hot water bath, Analytical Balance.

Principle:

By evaporating water content in milk under controlled conditions the total solids contents can be determined accurately.

Procedure:

- Weigh accurately the clean, dry empty dish with the lid. Pipette into the dish about 5 ml of the well mixed sample of milk and weigh quickly with the lid on the

dish. Place the dish uncovered on a boiling water-bath. Keep the base of the dish horizontal to promote uniform drying and protect it from direct contact with the metal of the water-bath. After at least 30 minutes, remove the dish, wipe the bottom and transfer to a well ventilated oven at 98 to 100^{O}C for about 3 hours, placing the lid by side of the dish. The bulb of the thermometer shall be just above the shelf carrying the dish. The dish shall not be placed near the walls of the oven.

1 After three hours, cover the dish and immediately transfer it to a desiccator. Allow cooling for about 30 minutes and weigh. Return the dish uncovered, and the lid to the oven and heat for one hour. Return to the desiccator, cool weigh as before. Repeat if necessary until the loss of weight between successive weighing does not exceed 0.5 mg. Note the lowest weight.

Calculation:

$$\text{Total Solids, percent by weight} = \frac{w}{W} \times 100$$

Where,

w = weight in gram of the residue after drying, and

W = weight in gram of the prepared sample taken for test.

iv) Phosphatase Test

Apparatus:

All purpose Lovibond comparator	Pipettes: 1.0 ml /5ml.
Standard discs-APTW or APTW/7	Graduated flask: 1000ml.
Two 25 mm. fused glass cells.	Measuring cylinder:100ml.
Water bath at 37 .5 +- 0.5degree centigrade	Test tubes

Chemicals Required:

Phosphatase dye: Dissolve 0.15 gm. of 4-Nitrophenyl phosphate disodium salt in 100 ml. buffer solution.

Buffer solution: Dissolve 3.5 gm of Na_2Co_3 and 1.5 gm $NaHCO_3$ to make 1 litre of solution in distilled water. Keep the buffer solution and the dye in a cool place.

Principle:

Raw milk contains an enzyme called alkaline-phosphatase. It is destroyed at the temperature necessary for efficient pasteurization. Pasteurisation indicate the destruction of pathogens. But when milk containing phosphatase is incubated with p-nitro-phenyl disodium orthophosphate, the liberated paranitro-phenol gives a yellow colour under the alkaline conditions of the test. The colour is a measure of the phosphatase content of the milk sample. Therefore, if phosphatase is present it indicate that the milk is not properly- pasteurised or has been contaminated after the heating process by raw milk.

Procedure:

Fill 5 ml solution of the phosphatase dye into test tubes marked at 10 ml . Add 1 ml of the milk to be tested, close the tubes with rubber stoppers and invert to mix. Prepare in the same way a blank form a boiled milk of the same type of that under test. Incubate all the tubes at 37 degree C. Read the yellow colour after 30 minutes, return to the bath, and take a second reading after incubation for a further 90 minutes. The yellow colour is read in a Lovibond all-purpose comparator on a resazurin stand, fitted with the disc calibrated in microgram *p*-nitrophenol. The blank is placed on the left of the stand and the sample on the right. Readings are taken by looking down on to the two apertures with the comparator facing a good source of north daylight; the disc is revolved until the sample is matched; readings falling between two standards are recorded to the nearest reading.

Interpretation of Results: *Disc Reading after 30 minutes incubation Interpretation*

0 or trace	**Properly pasteurized**
6	**Doubtful**
10 or ever	**Under pasteurized**

Disc Reading after 2 Hours

Incubation	*Interpretation*
0 to 10	**Properly pasteurized**
Over 10	**Under pasteurized**

The 30 minute test will reveal any serious fault in pasteurization, but to enable minor errors to be detected, readings shall be taken after further incubation for 90 minutes.

Check Your Progess - 3

1. What are the time-temperature combination for destruction of phosphatase?

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2. Does the destruction of the enzyme phosphatase indicate the destruction of pathogenic organisms and if so why?

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v) Determination of Ash Content in Milk

Apparatus:

Crucibles, Dessicator, Analytical Balance, Muffle Furnace, Electrical Heater

Principle:

Milk contains soluble substances containing salts like phosphates, citrates, sulphates, chlorides and bicarbonates of calcium, magnesium, potassium, sodium etc. Heating of milk at higher temperature decomposes organic matter and inorganic salts are left behind in the form of ash. Addition of neutralizers increase the ash contents in milk.

Procedure:

- Heat the crucible in order to remove any moisture from it.
- Cool the crucible in a dessicator to room temperature and weight it accurately.
- Weigh quickly and accurately about 10 g of milk in the weighed crucible.
- Evaporate the sample to dryness on a water bath, avoid spurting of the milk by using a thin glass rod drawn to a point and remove any particle adhering to the rod into the dish.
- Keep the evaporated milk sample in a muffle furnace at temperature not more than 550^{O}C until the ash is free from carbon. Normally it takes about 4 hours. Switch off power supply, wait for an hour before the crucibles are shifted to desiccator.
- Cool in a desiccator to room temperature and weigh quickly.

Observations:

Record the weights

- Weight of empty crucible = W g
- Weight of crucible with milk = W_1 g
- Weight of crucible after ashing = W_2 g
- Weight of milk taken = $W_1 - W$ g
- Weight of ash = $W_2 - W$ g

Calculation:

$$\text{Percentage of ash by weight} = \frac{W_2 - W}{W_1 - W} \times 100$$

vi) Determination of Protein in Milk by Formal Titration (Pyne's Method):

Apparatus:

Pipette – 10 ml, 2 ml and 1 ml graduated, Burette, flask 100 ml.

Reagents:

Neutral formalin, saturated potassium oxalate solution, N/10 sodium hydroxide, phenolphthalein indicator

Principle:

When formaldehyde is added to milk which has previously titrated against standard alkali to the end point of an indicator like phenolphthalein, it binds the amino groups of the milk proteins and release an equivalent amount of proteins (H-ions), which could be titrated against the alkali to the same end point. The amount of alkali used in the second titration is a measure of the amino groups originally present in the proteins.

Procedure:

- Pipette 10 ml of the well mixed sample of milk into a 100 ml of flask.
- Add 5 drops of phenolphthalein indicator
- Add 0.4 ml of saturated potassium oxalate and keep it aside for 2-4 minutes without disturbing.
- Titrate the milk against the standard alkali to its end point.
- Add 2 ml of neutral formalin and mix well.
- Titrate against the standard alkali to the same end point as before.
- Record the volume of alkali used in the second titration (V).

Calculation :

The percentage of protein in the given sample of milk = V × 1.7

Note:

If neutral formalin is not available, determine the formaldehyde acidity correction factor as given below and substract it from the second titration value and calculate the percentage of protein.

Pipette 2 ml of formalin in a 100 ml flask, add 10 ml of distilled water and 5 drops of phenolphthalein. Neutralize with N/10 sodium hydroxide to the usual end point. The volume of N/10 sodium hydroxide used gives the formaldehyde acidity correction factor.

II. Testing of Milk Powder

Moisture Content

Routine Method

Use Infrared Moisture Analyser and note the Moisture percent reading.

Gravimetric Method

Apparatus :

Refer Gravimetric Method for Milk.

Procedure:

- Place the dish and its lid in the oven at 102±2^{O}C for one hour. Transfer the dish and its lid from the oven to the desiccator. Allow it to cool to room temperature and weigh it.
- Mix thoroughly the milk powder sample and take approximately 1 gm. of the powder in the dish, cover the dish and weigh the covered dish accurately and quickly.
- Uncover the dish and put it with its lid in the oven at 102±2^{O}C for two hours.
- Replace the lid, transfer the covered dish to the desiccator, allow it cool to room temperature and weigh it accurately and quickly.
- Heat the uncovered dish and lid in the oven at 102±2^{O}C for further 1 hour, replace the lid, allow the covered dish to cool to room temperature in the desiccator and weigh it.
- Repeat the process until successive weighings do not differ by more than 0.5 mg. It is usually found that drying is complete after the first two hours.

Calculation:

$$\text{Moisture, percent by mass} = \frac{W_1 - W_2}{W_1 - W} \times 100$$

Where

M_1 = initial mass in g of the dish and lid with the material taken for analysis.

M_2 = the final mass in g of thed ish and lid with the material after drying; and

M = Mass in g of the empty dish

TITRATABLE ACIDITY

Apparatus:

Refer Determination of Titratable Acidity for Milk.

Procedure:

Weigh accurately 1 gm of the sample in a 100 ml beaker. Add 10 ml of warm distilled water and make a solution using a glass rod. Cool to room temperature. Add 1 ml phenolphthalein indicator and titrate against N/10 NaOH till a faint pink colour persists. **Calculate the acidity % as Volume of NaOH used X 0.9.**

ROSALIC ACID TEST

Procedure:

To 5 ml of reconstituted powder in a test tube, add 5 ml of alcohol. Add few drops of one percent (w/v) alcoholic solution of rosalic acid and mix. If neutralizer is present, a rose red colour appears whereas negative test shows only a brownish colouration.

Take 2 ml rosalic acid solution (0.05% in 60 : 40 alcohol and distilled water) in a test tube, add 2 ml of reconstituted powder. Rose-Red colour development indicates neutralizer presence in powder and formation of flakes indicates alcohol test positive.

SCORCHED PARTICLES

Procedure:

Reconstitute 10 grams of SMP or 13 grams of WMP into 100 ml. of distilled water in a flask. Observe the bottom of the flask in which powder is reconstituted after keeping undisturbed for some time. Filter the reconstituted milk through scorched particle tester and compare the filter pad with ADPI comparison card. Powder with scorched particles more than Disk B reading is rejected.

ASH CONTENT

Procedure:

Weigh 3 gm. of powder in a well-dried silica crucible and heat on a heater till no smoke comes out of the powder.

Keep it into a muffle furnace maintained at 550±20°C for three hours till grey ash formation. Switch off the muffle furnace and let the temperature fall. Transfer the crucible to a desiccator, cool completely and weigh. Heat the dish again at 550±20°C for 30 minutes. Cool the dish in a desiccator and weigh. Repeat this process of heating for 30 minutes, cooling and weighing until the difference between two successive weighings is less than one milligram. Record the lowest mass.

Calculate ash percentage as follows :

$$[A]\ \text{Ash \% by mass} = \frac{W2 - W}{W1 - W} \times 100$$

M2 = Weight of crucible with ash.

M = Weight of empty crucible.

M1 = Weight of crucible with powder.

$$\text{[B] Ash \% on dry matter basis} = \frac{\text{Ash\% by mass}}{(100 - \text{moisture\%})} \times 100$$

INSOLUBILITY INDEX

Apparatus:

Mixer, Centrifuge, 50 ml. graduated centrifuge tubes.

Procedure:

- The reconstituted temperature to be used in the insolubility index method will be 24^{O}C for spray-dried products.
- Take 13 gm in case of WMP and 10 gm in SMP. Reconstitute the powder in 100 ml distilled water and 24±0.2^{O}C. Add 3 drops of silicone anti-foaming agent and mix it thoroughly in the blender for 90 seconds. Further add 3 drops of silicone anti-foaming agent to the mixture and mix thoroughly with a spoon / spatula for 10 seconds. Pour the mixture into a centrifuge tube up to the 50 ml mark. Place the centrifuge tube in the centrifuge and rotate it for 5 minutes at 20 to 25^{O}C. Hold the centrifuge tube in a vertical position and remove the supernatant liquid. Add water upto 30ml mark in the centrifuge tube, completely disperse the sediment with the stirring rod and make up the volume upto 50 ml mark. Invert the centrifuge tube 5 times to mix its contents thoroughly. Place the centrifuge tube in the centrifuge and rotate it for 5 minutes at 20 to 25^{O}C.

Observation:

Remove the centrifuge tube from the centrifuge, hold the tube in a vertical position and read the volume of the sediment to the nearest 0.05 ml

FAT PERCENT IN WMP

Procedure:

- Take 10 ml of Gerber Sulphuric acid in milk Butyrometer.
- Weigh 1.69 gm of WMP in a 50 ml beaker and dissolve it in approximate 10 ml water, transfer carefully into the Butyrometer, add 1 ml Amyl alcohol, make the volume with water and centrifuge for 5 minutes.
- Record fat % by multiplying the Butyrometer reading with 20/3.

FAT PERCENT IN SMP

Procedure:

- Take 10 gm of SMP and reconstitute in 100 ml in distilled water.
- Take 10 ml of Gerber acid and 10.75 ml of reconstituted milk in the butyrometer.
- Add 1 ml Amly alcohol, and centrifuge for 5 min after mixing.
- Record Fat% by multiplying the Butyrometer reading with 10.

BULK DENSITY

Principle:

It is important in selection of packaging material. Weight remaining same, volume can affect the size of packing material, which in turn affect transportation. It can also influence consumer's perception about the product. Lost of packaging, transportation and storage is influenced bulk density.

Procedure:

Weight 30 gm of powder in 100 ml cylinder. Fix the cylinder in the frame of bulk density apparatus. Fix the apparatus for thirty strokes. Switch on the apparatus. The apparatus will take 30 strokes. Note the volume of the powder in the cylinder.

Calculate the density as follows:

$$\text{Density} = \frac{\text{Mass}}{\text{Volume}}$$

Mass = Weight of the Powder

Volume = Volume of powder after 30 strokes.

III. Testing of Butter

Method for the determination of Fat in White Butter

The method involves determination of moisture, curd and fat.

Determination of Moisture

Apparatus:

- Drying Oven maintained at 100±1^{O}C.
- Flat bottom moisture dish of Aluminium having 7 to 8 cm diameter and 2.5 cm depth.
- Glass Rods, Desiccator, Water Bath.

Procedure:

- Clean the dish and glass rod and dry in the oven maintained at 100±1^{O}C for at least one hour. Allow to cool to the room temperature in a desiccator and weigh the dish.
- Accurately 3 to 4 gm of the prepared butter sample weigh into the dish. Place the dish on a water bath for at least 20 minutes, stirring at frequent intervals until no moisture can be seen at the bottom (inside) of the dish. Wipe the bottom (outside) of the dish and transfer it to the oven maintained at 100±1^{O}C and keep it for 90 minutes. Allow the dish to cool in the desiccator as before and weigh. Heat the dish again in the oven for 30

minutes. Repeat the process of heating, cooling and weighing until the difference between two consecutive weights does not exceed 1 mg. Record the lowest weight.

- Preserve the residue for the determination of curd.

Calculation:

$$\text{Moisture, percent by weight} = \frac{100(W_1 - W_2)}{W_1 - W}$$

Where

W_1 = weight in gm of the dish with the material before heating to constant weight,

W_2 = weight in gm of the dish with the material after heating to constant weight, and

W = weight in gm of the empty dry dish.

Determination of Curd

Apparatus:

Glass Funnel - with folded 12.5 cm Whatman No.1 filter paper

Flat bottom flask - 250 ml capacity

Desiccator

b) Reagents:

Petroleum ether - Boiling range 40^0 - 60^OC.

Procedure:

- Dry, cool and weigh glass funnel with folded 12.5 cm filter paper. Melt the residue in the moisture dish from the moisture determination, add 25 to 50 ml of petroleum ether and mix well. Place the funnel with the filter paper, wet the filter paper with petroleum ether and decant the fatty solution from the dish into the filter paper, leaving the sediment in the dish. Add 20 to 25 ml of petroleum ether twice and decant the fatty solution into the filter paper, collect the filtrate in a clean, dried, tared 250 ml flat bottom flask containing a glass bead. With the aid of a wash bottle containing petroleum ether wash all the fat and sediment from the dish into the filter paper.

- Finally wash the filter paper until free from fat, collecting all the filtrate in the flask. Preserve the filtrate for the determination of fat.

- Dry the filter funnel in the oven maintained at 100 ± 1^OC for at least 30 minutes. Cool in the desiccator and weigh. Repeat drying, cooling and weighing until the loss of weight between the consecutive weighings does not exceed 1 mg.

Calculation:

$$\text{Curd, percent by weight} = \frac{100(W_1 - W_2)}{W}$$

Where

W_1 = weight in gm of the filter funnel with residue

W_2 = weight in gm of the filter funnel alone, and

W = weight in gm of the sample

Determination of Fat

Procedure:

Distill off the solution of fat in petroleum ether collected in a tared flask. After removing all traces of solvent, dry the flask containing fat in an oven maintained at 100±1^{O}C for one hour, cool in a desiccator and weigh. Continue the drying, cooling and weighing until the loss of weight between consecutive weighings does not exceed 1 mg.

Calculation:

$$\text{Fat, percent by weight} = \frac{100(W_1 - W_2)}{W}$$

Where

W_1 = weight in gm of the 250 ml flask with dried fat

W_2 = weight in gm of the empty flask, and

W = weight in gm of the sample

Titratable Acidity :

Weigh accurately about 20 gm of the butter sample in a dry 250 ml conical flask. Add 90 ml of hot, previously boiled water and shake the contents. While still hot titrate with 0.02 N (N/50) sodium hydroxide, using 1 ml of phenolphthalein indicator.

Calculate acidity percent (as lactic acid):

$$\text{Percent by weight} = \frac{9XNXV}{W}$$

Where

N = normality of sodium hydroxide solution,

V = Volume of sodium hydroxide, and

W = Weight in gram of the sample.

Analysis of Salt in Table Butter

Principle:

The butter is melted in hot water and the chloride is titrated with a solution of silver nitrate using potassium chromate as indicator.

Apparatus:

Conical flask- 250 ml capacity.

Burette – 50- ml graduated to 0.1 ml.

Reagents:

Calcium carbonate (analytical grade, free from chloride)

Potassium chromate indicator (5 % (W/V) solution in water)

Standard silver nitrate solution – 0.1N

Procedure:

Weigh accurately about 5 gm of the sample into the 250 ml conical flask. Carefully add 100 ml of boiling water. Allow to stand with occasional swirling for 5 to 10 minutes. After cooling to 50 to 55ºC (Titration temperature), add 2 ml of potassium chromate solution. Mix by swirling. Add about 0.25 gm of calcium carbonate and mix by swirling. Titrate at 50 to 55ºC with standard silver nitrate solution while swirling continuously, until the brownish colour persists for half a minute. Carry out a blank test with all the reagents in the same quantity except the sample material. The maximum deviation between duplicate determination should not exceed 0.02% of Na Cl.

Calculation:

$$\text{Sodium chloride, percent by weight} = \frac{5.85N(V1 - V2)}{W}$$

Where

N = normality of silver nitrate solution

V1 = volume of silver nitrate in the sample titration

V2 = volume of silver nitrate in the blank titration, and

W = weight in gm of the sample.

IV. Testing of Ice cream

Method for Determination of Fat (Gerber Method):

Apparatus:

Analytical Balance, Hot Air Oven, Auto titrator, Gerber Centrifuge, Incubator and Water Bath.

Reagents:

Sulphuric acid (Specific Gravity 1.807 to 1.812) corresponding to a concentration of 90 to 91% by mass.

Amyl alcohol (Specific Gravity 0.810 to 0.812) conforming to grade 1of IS:360:1964.

Procedure:

Take 10 ml Gerber Acid in a clean and dry ice-cream butyrometer (20% scale).

Add about 1-2 ml distilled water carefully from the walls of the butyrometer to make a separate layer of water over the acid.

Take about 5gm of the sample (3gms. in case of chocolate mix/ chocolate ice cream) accurately weighed (it is convenient to weigh by difference) in the butyrometer.

Add 2 ml Amyl alcohol.

Maintain the level in the butyrometer with warm distilled water and close the butyrometer with a lock stopper.

Shake the butyrometer vigorously without inverting until the contents are thoroughly mixed and no white particles are seen. Then invert the butyrometer few times.

Centrifuge the butyrometer at 1400 ± 70 RPM for 3 minutes.

Transfer the butyrometer keeping the stopper down-wards into a water bath at a temperature 65± 2°C for 3 minutes, and then take out it and note the fat % from the graduated scale Calculate fat % for 5 gm. of sample.

Method for Determination of Protein (Pyne's Method)

Chemicals Required:

Phenolphthalein indicator solution.

Potassium oxalate solution: Saturated.

Formaldehyde: 40 % concentrated and neutral phenolphthalein.

Standard NaOH solution: N/10

Testing:

Take 10 gm. of the sample accurately weighted in a conical flask (it is convenient to weigh by difference). Add 50 ml distilled water to it.

Add 1 ml. of phenolphthalein indicator solution to it followed by 0.4 ml. saturated potassium oxalate solution mix the contents of the flask and set it aside for 2 minutes.

Neutralize the contents of the flask with standard N/10 NaOH solution to a pink coloured end point.

Add 2 ml neutralized formaldehyde and again titrate with standard NaOH solution to the same pink shade. Note the volume of NaOH solution used (V).

Calculation:

Protein %. = V X 1.7

Method for Determination of Titratable Acidity

Chemicals Required:

Phenolphthalein indicator solution.

Standard sodium hydroxide solution (N/10).

Procedure:

- Weight accurately about 10gm (weight by difference) of the sample into a 100 ml flask.
- Add 50ml. of boiled and cooled distilled water to it. Mix properly.
- Add 1ml. Phenolphthalein indicator solution and titrate against standard sodium hydroxide solution (N/10) to a light pink colour end point.
- Note the volume of NaOH solution used and calculate the titratable acidity as follows.
- Titratable Acidity (as Lactic acid) % by weight $= \frac{9NV}{W}$

Where,

N = Normality of the standard NaOH solution.

V = Volume of the standard NaOH solution.

W = Weight of the sample taken.

Method for Determination of Total Solids (Gravimetric method)

Apparatus: Shallow Flat bottom dishes of aluminum alloy having 7-8cm diameter and about 105cm weight with a lid. Hot Air oven ($100^0 +1^0$ C) water bath and Analytical balance.

Procedure:

- Weigh accurately clean and dry metal dish (dried & cool in a desiccator).
- Take about 2 gm. (weight by difference) of sample in it and weigh it again.
- Add 2-3 ml. of distilled water to it, spread the sample properly in the dish and dry it on a hot plate carefully.
- Transfer the dish in a well-ventilated hot air oven at 100±1°C for one and a half-hour.
- Transfer the dish in a desiccator for cooling and then weigh again.

Calculation:

From the loss in weight calculate the total solids % by weight of the material taken:

$$\text{Total solids \% by weight} = \frac{W_3 - W_1}{W_1 - W} \times 100$$

Where,

W1 = Weight of empty dish.

W2 = Weight of dish with sample (before drying)

W3 = Weight of dish with sample (after drying)

Method for Phosphatase Test

Chemicals Required:

Phosphatase dye: Dissolve 0.15 gm. of 4-Nitrophenyl phosphate disodium salt in 100ml. buffer solution.

Buffer solution: Dissolve 3.5gm. of Na_2CO_3 and 1.5gm. $NaHCO_3$ to make 1 litre of solution in distilled water. Keep the buffer solution and the dye in cool place.

Procedure:

- Take plain mix 1 ml each in two test tubes.
- Boil the contents of one of the test tubes to be used as control (reference).
- Add phosphatase dye 5 ml in each test tubes and mix the contents thoroughly.
- Plug the test tubes with cotton or rubber stoppers.
- Place the test tubes in an incubator/water bath maintained at a temperature 37(±1)°C and note for any change in colour. Development of yellow colour in less than two and a half-hours time indicates a positive test & no colour change indicates negative test.

Determination of Titratable Acidity (Candy Mix)

Chemicals Required:

Phenolphthalein indicator solution.

Standard sodium hydroxide solution (N/10).

Procedure:

Weight accurately about 10gm (weight by difference) of the sample into a 100ml. flask.

Add 1ml. phenolphthalein indicator solution and titrate against standard sodium hydroxide solution (N/10) to a light pink colour end point.

Note the volume of NaOH solution used and calculate the titrable acidity as follows.

$$\text{Titrable Acidity (as citric acid) \% by weight} = \frac{0.064NV}{W}$$

Where N = Normality of the standard NaOH solution.
V = Volume of the standard NaOH solution.
W = Weight of the sample taken.

Determination of Total Solids (Candy Mix)

Procedure:

- Take about 1 gm. Sea sand in an empty aluminium dish dried in a hot air oven and place it again in the oven for 30 min. to eliminate moisture.

- Take out the dish containing sand and cool it in a desiccator.
- Take about 2 gm. (weight by difference) of sample in it and weigh it again.
- Spread the sample properly in the dish and dry it on a hot plate carefully.
- Transfer the dish in a well-ventilated hot air oven at 102(±1)°C for one and a half-hour.
- Transfer the dish in a desiccator for cooling and then weigh again.

Calculation:

From the loss in weight calculate the total solids % by weight of the material taken

$$\text{Total solids \% by weight} = \frac{W_3 - W_1}{W_1 - W} \times 100$$

Where

W1 = Weight of empty dish.

W2 = Weight of dish with sample (before drying)

W3 = Weight of dish with sample (after drying)

V. Testing of Paneer/Hard Cheese

Determination of Moisture

Procedure:

- Weigh accurately about 2 gm of shredded paneer into the previously dried and weighed dish. Mix the material uniformly with 4 ml of hot distilled water with the help of a small glass rod. Wash off the particles of material adhering to the glass rod by pouring an additional 1 ml of hot distilled water. Heat the dish containing the material after uncovering in the oven maintained at 102 ± 1^{O}C for 4 hrs.
- Cool the dish in the desiccator and weigh with cover on.
- Replace the dish in the oven for 30 minutes until the difference between the two consecutive weighings is less than one milligram.
- Record the lowest weight.

Calculation:

$$\text{Moisture percent by mass} = \frac{100(W_3 - W_1)}{W_1 - W} \times 100$$

Where

W = Mass in gm of empty dish

W_1 = Mass in gm of dish with sample before drying

W_2 = Mass in gm of dish with sample after drying.

Determination of Fat

Reagents:

Gerber sulphuric acid

Amyl Alcohol

Procedure:

- Take 10 ml of gerber acid into butyrometer.
- Transfer accurately 1.69 gm of shredded / meshed paneer to the butyrometer.
- Add 1 ml of amyl alcohol with the help of tilt measure.
- Make up the volume in butyrometer by adding distilled water.
- Close the butyrometer with rubber - stopper and mix the contents by shaking until the entire sample has been digested.
- Place the butyrometer in the centrifuge, balance the machine and centrifuge for 5 minutes.
- Transfer the butyrometer in water bath at 65 ± 2^{O}C for 5 minutes.
- Adjust the fat column within the scale and take reading.

Calculation:

Percent Fat =Butyrometer reading $X\frac{20}{3}$

Percent Fat by Mass (on dry basis) = $\frac{\text{Pr esent Flat}}{100 - \text{Percent Moisture}} \times 100$

Determination of Titratable Acidity

Reagents:

Standard Sodium hydroxide solution (N/10).

Phenolphthalein indicator (0.5%)

Standard hydrochloric acid (N/10)

Procedure:

- Weigh 2 gms of the meshed paneer into the flask.
- Add 3 ml of boiling distilled water and mix well with a rod and further add 17 ml of boiling water over the rod.
- Cool to room temperature and add 10 ml of N/10 NaoH and 1 ml phenolphthalein.
- Titrate against N/10 HCl till disappearance of pink colour.

Calculation:

$$\text{Acidity (Percent lactic acid)} = \frac{10 - V}{W} \times 0.9$$

Where,

V = Volume in ml of standard acid used in titration.

W = Weight in gm of sample.

VI. Testing Of Ghee

Determination of Moisture

Apparatus:

- Drying Oven maintained at 105±1^{O}C.
- Flat bottom moisture dish of Aluminium having 7 to 8 cm diameter and 2.5 cm depth.
- Glass Rods, Desiccator, Water Bath.

Procedure:

- Clean the dish and glass rod and dry in the oven maintained at 105±1^{O}C for at least one hour. Allow to cool to the room temperature in a desiccator and weigh the dish.
- Accurately weigh into the dish 10 gm of the prepared ghee sample and transfer it to the oven maintained at 105±1^{O}C and keep it for 60 minutes. Allow the dish to cool in the desiccator as before and weigh. Heat the dish again in the oven for 30 minutes. Repeat the process of heating, cooling and weighing until the difference between two consecutive weights does not exceed 1 mg.
- Preserve the residue for the determination of curd.

Calculation:

$$\text{Moisture, percent by weight} = \frac{100\ (W_1 - W_2)}{W_1 - W}$$

Where

W_1 = weight in gm of the dish with the material before heating to constant weight,

W_2 = weight in gm of the dish with the material after heating to constant weight, and

W = weight in gm of the empty dry dish.

Free Fatty Acids Percent as Oleic Acid

Reagents:

Standard Sodium Hydroxide (N/10)

Phenolphthalein Indicator (0.5%)

95 percent rectified spirit (Neutralised with NaOH using Phenolphthalein as indicator).

Procedure:

- Weigh exactly 10 gm of ghee in a dry 250 ml conical flask.
- To this add 50 ml of neutralised rectified spirit and heat to boiling on a hot plate.
- Titrate against N/10 NaOH solution using Phenolphthalein till pink colour persists.

Calculation:

$$\text{Percent FFA (as Oleic Acid)} = \frac{V \times 2.82}{W}$$

Where,

V= Volume of NaOH used in titration

W= Weight in gm of sample.

Butyro Refractometer Reading

Apparatus:

Refractometer (Digital)

Reagent:

Rectified Spirit, Glass distilled water

Procedure:

Clean the prism of digital refractometer gently. Check the value of glass distilled water at 40°C. It should be zero. Dry the prism properly and put few drops of melted ghee and take reading at 40°C. Instrument will show refractive index. Convert R.I. reading into B.R. with the help of chart. After completion of work, clean the prism with rectified spirit and glass distilled water by using soft tissue paper. Switch off the instrument.

In case BR meter is used direct reading is taken.

Ghee

RI (1.4524 to 1.4545)	B.R.(40 to 43)
1.4524	40.0
1.4525	40.1
1.4526	40.3
1.4527	40.4
1.4528	40.6
1.4529	40.7
1.4530	40.9
1.4531	41.0
1.4532	41.1
1.4533	41.3

1.4534	41.4
1.4535	41.5
1.4536	41.7
1.4537	41.8
1.4538	42.0
1.4539	42.1
1.4540	42.3
1.4541	42.4
1.4542	42.5
1.4543	42.7
1.4544	42.8
1.4545	43.0
1.4548	43.5
1.4552	44.0
1.4555	44.5
1.4558	45.0

Determination of Reichert – Meissl Value (R.M.Value)

Reagents :

- Glycerine (analytical reagent grade)
- Concentrated sodium hydroxide solution 50 percent (weight/weight)
- Dilute sulphuric acid solution:- Approximately 1N (27.7 ml in one lit water)
- Sodium hydroxide solution :- N/10
- Phenolpthlein indicator :- Dissolve 0.1 gram of phenoppthalein in 100 ml of ethyl alcohol.
- Ethylalcohol:- 90% by volume and neutral to phenolphthalein .

Procedure :

- Weigh accurately 5(+-0.01) gram filtered oil or fat sample in to clean, dry, 300ml distilling flask.
- Add 20 gram of glycerine and 2 ml of concentrated sodium.hydroxide (50% W/W) solution:
- Heat the flask with swirling over flame until completely saponified and mixture becoming perfectly clear.
- Cool the content slightly and add 90 ml of boiling distilled water, which has been

boiled for about 15 minutes. If the solution is not clear repeat the test with fresh sample.

- Add about 0.1 gram of pumice stone or 4-5 glass beeds, and 50 ml of dilute sulphuric acid solution.
- Connect the flask to the distillation apparatus and heat very gently until the liberated fatty acids melt and separate.
- Set the flame so that 110 ml of distillate shall be collected within 19 to 21 minutes.
- When the distillate exactly reaches the 110 ml mark on the flask, remove the flame and quickly replace the flask by 25 ml measuring cylinder stopper the graduated flask and without mixing placed it in a water bath maintained at 15 degree for 10 minutes so that the 110 ml graduated mark is 1 CM below the water bath, dry the outside and mix the content gently by inverting the flask 4 or 5 times without shaking.
- Filter the liquid through a dry, 9cm whatman no.4 filter paper.
- Pipette 100 ml of the filtrate and add 5 drops of the phenolphtalein solution.
- Run a blank test without fat, but using the same quantities of the reagents.
- Calculation
- Reichert-meissl value = (A-B) X N X 11 or (A-B) x 1.1

A= Volume in ml of standard sodium hydroxide required for the test.

B= Volume in ml of the standard sodium hydroxide required for the blank.

N= Normality of standard sodium hydroxide solution

VII. Testing of FLAVOURED MILK (UHT Processed)

Determination of Fat

Follow same as in liquid milk

Determination of Total Solids

Follow same as in liquid milk

Determination of Acidity

Follow same as in liquid milk .Weigh exactly 10 gm of flavoured milk instead of 10 ml as mentioned in milk.

VIII. Testing of STERILIZED CREAM

Determination of Fat

Procedure:

- Take 10 ml of gerber sulphuric acid into cream butyrometer (0-40%) with the help of tilt measure.

- Weigh 5gm of cream into the butyrometer. To this add 1 ml amyl alcohol with the help of tilt measure.
- Close the butyrometer with rubber stopper and mix the content by shaking until all the curd has been digested.
- Place the butyrometer in the centrifuge, balance the machine and centrifuge for 5 minutes. Transfer the butyrometer in water-bath at 65 ± 2^{O}C for 5 minutes.
- Adjust the fat column within the scale and take reading.

Determination of Acidity

Reagents:

Standard Sodium Hydroxide (N/10)

Phenolphthalein Indicator (0.5%)

Procedure:

- Weigh exactly 10 gm of cream in a dry 250 ml conical flask.
- To this add 20 ml of hot distilled water and titrate against N/10 NaOH solution using Phenolphthalein till pink colour appears.

Calculation:

$$\text{Percent Acidity} = \frac{V \times 0.9}{W}$$

Where,

V = Volume of NaOH used in titration

W = Weight of sample in gm

IX. Testing of LASSI

Determination of Fat:

Follow same as per Dahi (As per11.1).

Determination of Total Solids

Follow same as in liquid milk (As per 4.3.3).

Determination of Acidity

Follow same as in liquid milk (As per 4.3.10).

Weigh exactly 10 gm lassi instead of 10 ml as mentioned in milk.

In acidometer, select curd.

X. Testing of for CURD (DAHI)

Determination of Fat

Procedure:

- Weigh 100 gm of the curd in beaker
- Add 5 ml of strong ammonia to the weighed sample and shake well to make it homogeneous.
- Pipette out 10.75 ml of well mixed sample of Dahi and transfer it to the butyrometer.
- To this add 1 ml amyl alcohol with the help of tilt measure.
- Close the butyrometer with rubber stopper and mix the content by shaking until all the curd has been digested.
- Place the butyrometer in the centrifuge, balance the machine and centrifuge for 5 minutes.
- Transfer the butyrometer in water-bath at 65 ± 2^{O}C for 5 minutes.
- Adjust the fat- column within the scale and take reading.
- Multiply the result obtained by the dilution factor (in this case 1/20) and add the same to the obtained result to get the actual result.

Determination of Acidity

- Follow same procedure as in liquid milk
- Weigh exactly 10 gm dahi instead of 10 ml as mentioned in milk.
- In acidometer, select curd.

XI. Testing of WATER

Hardness:

Take 50 ml of water sample in a 250 ml conical flask, add one powdered total hardness indicator tablet or 2 to 3 drops of 0.5% alcoholic Eriochrome black-T solution (indicator) to it and shake well. Now add 2 ml of ammonia buffer solution and shake well. Titrate this mixture with N/50 EDTA solution.

Note the volume, in ml, of EDTA solution used and multiply it by 20. The product is the hardness, in ppm as calcium carbonate, of the water sample.

pH:

Use pH paper to check the pH

Sulphite Ions (For Boiler Water):

Take 50 ml of boiler water in a conical flask, neutralize it with about 2 ml of 50% HCl solution and then add 1 ml of 1% starch solution and titrate it with iodate-iodide solution taking blue color as the end point.

Note the volume of iodate-iodide solution used and multiply with 16. The product is the ppm of Sulphite ions.

Phosphate Ions (For Boiler Water):

To 1 ml of filtered boiler water add 1 ml of HNO_3 solution and a pinch of ammonium molybdate crystals and shake well.

Yellow colour development indicates presence of Phosphate ions.

Residual Chlorine in Water (For Processing):

It is tested by TOLIDINE method, generally, and also by CHLOROTEX method. Acceptable limit is 0.1 to 0.2 ppm.

Tolidine Method:

To 50 ml water in a measuring cylinder add 1 ml of O-tolidine solution, observe the colour and record in the register.

Chlorotex Method :

Take 5 ml chlorotex reagent in a cylinder and add 50 ml water. Compare the colour with the standard colour chart given on the chlorotex reagent bottle.

Preparation of Chemical solutions / Reagents:

Alcoholic KOH: (Dissolve 7 gms. KOH pallets in 10 ml distilled water and make the volume to 250 ml by adding rectified spirit).

DMAB (p-dimethylamine benzaldehyde): In a liter of Rectified spirit dissolve 16 gms p-dimethylamine benzaldehyde powder and add 100 ml of concentrated HCl.

Ethylene Diamine Tetra Acetic Acid (N/50 E.D.T.A.): Dissolve 3.72 gms of EDTA powder in distilled water and make volume to 1 liter.

Eryochrome Black t: Dissolve 0.5 gm eryochrome Black T in 100 ml rectified spirit.

FERRIC chloride solution (1%): Dissolve 1 gm of Ferric chloride crystals in distilled water and make volume to 100 ml.

Hydrochloric acid (50%) : Dilute 50 ml of concentrated hydrochloric acid with 50 ml of distilled water.

Hydrochloric Acid Solution (N/10) : Dilute 25 ml concentrated HCl to 2.5 liter in distilled water (10 ml concentrated HCL in 1 litre distilled water).

Iodine Solution (1%): Dissolve 1 gm Iodine reagent and 1 gm KI in distilled water and make volume to 100 ml.

Iodate Iodine solution: Dissolve 0.71 gm of potassium iodate (KIO_3), 7.0 gm of potassium iodate (KIO_3), 7.0 gm potassium iodide (KI) and 0.5 gm Sodium Bicarbonate ($NaHCO_3$) in distilled water and make the volume to 1 liter.

Oxalic Acid 0.1N : Dissolve 6.30 gm in 1000 ml of distilled water.

Phenolphthalein solution (0.5%) : Dissolve Phenolphthalein powder, 5 gms in 50% alcohol (500 ml) and then add 500 ml distilled water make up the volume 1 litre.

Potassium iodide solution (7%) : Dissolve 7 gms. of potassium iodide powder in 100 ml. distilled water.

Potassium chromate (10%) : Dissolve 10 gms. of potassium chromate powder in 100 ml of distilled water.

Phosphate Dye : Mix 3.5 gms Sodium Carbonate and 1.5 gms sodium Hydrogen Carbonate in 1000 ml distilled water to prepare phosphate buffer solution. In 100 ml of this buffer mix 0.15 gm of 4-Nitrophenyl Disodium salt.

Potassium Hydroxide 0.1N : Dissolve 5.61 gm in 1000 ml of distilled water.

Potassium Chromate 0.1N : Dissolve 4.903 gm in 1000 ml of distilled water.

Paraphenylene diamine hydrochloride solution (2%) for H_2O_2 test : Dissolve 2 gm of Paraphenylene diamine hydrochloride powder in 100 ml. distilled water.

Rosalic acid solution (0.05%) : Dissolve 2.5 gms of Rosalic acid powder in 5 litres of 60% alcohol (to 3.160 litres of rectified spirit and 1.840 litres of distilled water). Density 0.91

Rosaniline Acetate Solution (Stock Solution): Dissolve 0.12 gm of rosaniline acetate in approximately 50 ml of rectified spirit containing 0.5 ml of glacial acetic acid. Make up to 100 ml with rectified spirit.

Rosaniline Acetate Solution (Bench Solution) : Dilute 1 ml of the stock solution to 500 ml with a mixture of rectified spirit and distilled water in equal proportions by volume.

Resorcinol Solution for sugar test : 0.1 gram Resorcinol dissolve in HCl (1:2) i.e. 35 ml concentrated HCl added to 70 ml distilled water.

Sodium Hydroxide solution (N/10 NaOH) : Dissolve 4 gms. of Sodium Hydroxide pellets in distilled water and make up the volume to 1 liter. Standardized with N/10 oxalic acid.

Sodium Hydroxide Solution (2%) : Dissolve 2 gms of NaOH pellets in 100 ml of distilled water.

Starch Solution (1%) : Dissolve 1 gm of starch powder in 100 ml of warm distilled water and cool.

Silver Nitrate solution (0.1341%) : Dissolve 0.1341 gm of $AgNO_3$ powder in distilled water and make volume to 100 ml. (For Salt Test)

Silver Nitrate N/10 (To check chloride in water) : Dissolve 1.698 gm silver nitrate in distilled water and make up the volume upto 100 ml.

Sodium Carbonate 0.1N : Dissolve 5.3 gm. in 1000 ml. of distilled water.

Alcoholic KOH Solution (For Mineral Oil Test) : Dissolve 28 gms of KOH in 1 liter distilled alcohol.

Check Your Progress – 4

1. Why ask content of milk is determined?

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2. What is the Principle of determination of protein in milk by formal titration method?

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3. What is the importance of bulk density of dried?

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4. What is the objective of determination of acid value and RM Value of ghee?

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7.3 LET US SUM UP

Test methods most commonly used are included in this unit. The students are advised to refer the BIS methods for more understanding. Since testing is very critical for business utmost care is required in establishing a proper laboratory and employing skilled personnel for running the same in the dairies.

7.4 KEY WORDS

Platform tests **:** when milk is received in the dairy, samples are taken and tested in a laboratory, situated near the reception area. The samples are kept on work bench or concrete platform for testing. Hence the name.

B.R. Reading : a special value for Ghee. Legal requirement.

R.M. Value : a special value to know the purity of Ghee.

Titratable Acidity : a value required in the keeping quality of milk and milk product. Determine the shelf life of the product.

Under each method of testing apparatus, reagents and principle are explained which include key words. Hence more words are not included here.

7.5 SOME USEFUL BOOKS

IS 1165:2002	Milk Powder (fourth revision)
IS 1223 2001	Apparatus for determination of fat by gerber method (second revision)
IS 1224 (PT 1) 1977	Determination of milk fat by the gerber method : Part 1 Milk (first revision)
IS 1224 (PT 2) 1977	Determination of milk fat by the gerber method : Part 2 Milk Products
IS 1479 (PT 1): 1960	Methods of test for dairy industry : Part 1 Rapid examination of milk
IS 1479 (PT 1): 1960	Methods of test for dairy industry : Part 1 Rapid examination of milk
IS 1479 (PT 2): 1961	Methods of test for dairy industry : Part 2 Chemical Analysis milk
IS 1656 :1997	Milk cereal based weaning foods (third revision)
IS 1806:1975	Malted milk foods (first revision)
IS 2311:1973	Fat extraction apparatus for milk and milk products (first revision)
IS 2785:1979	Natural cheese (hard variety), processed cheese, processed cheese spread and soft cheese (first revision)
IS 2802 1964	Ice Cream
IS 4709:1968	Flavoured milk
IS 4883:1980	KHOA (first revision)
IS:4884:1968	Sterilized cream
IS 5162:1980	CHHANA (first revision)
IS 9070:1979	Method of determination of fat in cheese by Van Gul method

IS 9532:1980	Chakka and Shrikhand
IS 9585:1980	Lactometers
IS 9617:1980	DAHI
IS:10083:1982	Method of test for determination of SNF (solids not fat) In milk by the use of lactometers
IS 10484:1983	PANEER
IS 10501:1983	KULFI
IS 11202:1999/	Method for determination of lactic acid and lactate content
ISO 3495:1997	in milk powder and similar products
IS 11622:1986	Method for determination of total solids content in condensed milk
IS 11623:1986	Method for determination of moisture content in milk Powder and similar products
IS 11721:1986	Method for determination of fat content in milk powder and ISO 1737:1985 similar products (reference method)
IS 11766:1986	Method for determination of titratable acidity in milk
ISO 6092:1980	powder and similar (routine method)
IS:12299:1998	Dairy Whitener
IS:12333:1997	Method for determination of total solids content in milk,
ISO 6731:1989	cream and evaporated milk (reference method)
IS:12759:1989	Dried milk and dried milk products for determination of
ISO 8156:1987	Insolubility index
IS:12760:1989	Dried milk - determination of sodium and pottasium
ISO 8070:1987	contents by flame Emission Spectrometric method
IS:12898:1989	Yoghurt

IS 13334(PT 1):1998	Skim Milk Powder – Standard Grade
IS 13334(PT 2):1992	Skim Milk Powder – Extra Grade
IS 13500:1992	Spray dried milk powders – Scorched particles –Determination
IS:13688:1999	Pasteurized milk
IS:13689:1992	Butter Oil (Butter fat)
IS:13690:1992	Pasteurized butter
IS:14433(PT 1):1997	Infant milk substitutes – Specification : Part 1 Milk protein based
IS:14542:1998	Partly skimmed milk powder

Manual in Dairy Chemistry published by NDRI, Bangalore

Testing manual by DGHS – Govt. of India.

7.6 ANSWERS TO CHECK YOUR PROGRESS

Your answer should include the following points.

Check Your Progress – 1

1. Quick determination of developed acidity in milk?

2. Alcohol test is done to determine the heat stability of milk. If milk is not sufficiently stable it get coagulated when mixed in 1:1 ratio

3. In the manufacture of UHT milk, evaporated milk, sweetened condensed milk and milk powder/

4. a) Natural acidity-proteins phosphate citrate.

   b) Developed acidity - lactic acid.

5. Developed acidity make the milk unfit for human consumption and it get coagulated.

Check Your Progress – 2

1. Preservative to prevent the growth of microorganisms and developed acidity and to extend the shelf life.

Adulterants – For economical gains

2. Adulterant.

3. Sugar is added to increase the desity or specific gravity oil mineral is added to indicate higher milk fat content.

Check Your Progress – 3

1. 62.8^{O}C for 30 Min/71.7^{o} for 15 seconds.

2. Yes, the temperature of inactivation of alkaline phosphatase enzyme in milk is slightly high than the temperature at which most stable pathogen (microbacter tuberclosis) is destroyed.

Check Your Progress – 4

1. Ash content is determined to ascertain wheather neutralizers are added to milk or not or if the milk is of late lactation.

2. Formalin react with amino groups of proteins and release the equivalent No of H+ ions which is proportional to the protein content in milk.

3. Effect the size of container transpiration cost and storage space which are indirectly proportional to bulk density hence affect the cost of production.

4. Acid value of ghee indicate its quality higher the acid value poor is the quality of ghee, R.M. value indicate the purity or adulteration of ghee with other fats.