R35·12

D.P.H.

Milk.

Milk is the physiological secretion of the mammary gland of mammals. It is an aqueous fluid containing fat in suspension in fine particles, proteins in true or colloidal solution and lactose, mineral salts and traces of certain other constituents in true solution. The approximate analyses of the milk of a number of different mammals is given in the following table:

Mammal	Fat	Protein	Sugar	Ash	Water	Sp. Gr.
Woman	3.3	1.5	6.8	0.2	88.2	1031
Cow	3.9	3.4	4.75	0.75	87.2	1030
Ass	1.02	1.79	5.5	0.42	91.17	
Mare	2.5	2.61	5.5	0.5	88.8	
Goat	4.2	3.62	4.0	0.56	87.54	1030
Ewe	5.3	7.1	4.2	1.0	82.27	1040
Elephant	19.87	3.09	3.13	0.85	67.85	
Sow	4.55	7.23	3.13	1.03	84.04	
Camel	3.07	4.0	5.59	0.77	86.57	
Bitch	9.57	9.91	3.19	0.73	76.6	1035
Buffalo	4.63	4.35	4.22	0.76	86.07	
Cat	3.33	9.55	4.91	0.58	81.63	

All milks have a somewhat similar composition but individual differences exist, e.g., the milk of sheep has a high specific gravity and a large amount of solid matter and the milks of the cat and the dog contain a large proportion of albuminous solid. The milks of woman and of the ass are peculiar in that their casein content is not precipitated under the usual conditions on souring or by the addition of acetic acid.

Analytical Details.

Sampling - Before carrying out an analysis adequate precautions must be taken to ensure that a true sample is obtained, e.g., care should be taken to make sure that any fat sticking to the side of the vessel is detached and the whole evenly emulsified. For a complete analysis 250-500 cc. ($\frac{1}{2}$ - 1 pint) is required. 50-60 cc. will suffice for a fat determination only.

Specific Gravity - May be determined with (a) a lactometer, (b) a specific gravity bottle, or (c) Westphal's balance.

(a) A hydrometer is a weighted float with a long neck carrying a scale, graduated so that the specific gravity of any fluid in which it freely floats is given by the graduation mark at the surface of the fluid. A lactometer is a hydrometer used for determining the specific gravity of milk.

(b) A specific gravity bottle is a bottle that holds exactly 50 cc. of a liquid at 15.5°C. when its stopper, which has a capillary channel running through it to allow excess fluid to ascape, is inserted. The specific gravity of milk is given by the ratio of the weight of this quantity of milk to the weight of the same quantity of water at 15.5°C.

Westphal

(c) Westphal's balance - This is a balance that carries a suspended float at one end of the beam. This float is freely suspended but totally immersed in the milk and the beam balanced by placing special weights on the same half of the beam that carries the float. Upon this beam is a scale and four weights are used, which differ from each other by multiples of ten. The respective positions on the scale of these weights from the heaviest to the lightest give the four figures of the number representing the specific gravity. For a milk of S.G. 1032, the heaviest weight would be in position 1, the 3rd weight* and the lightest in position 2 at balance. The specific gravity for cow's milk at 15.5°C. generally falls between 1028-1034. A low specific gravity means that either the milk contains an excess of fat or that water has been added. A high specific gravity may indicate that cream or fat has been removed.

* in position 3

Total Solids - For the analysis of milk the constituents are expressed as percentages by weight. The total solids of milk is that weight remaining when the entire water content but nothing else of 100 g. of milk are evaporated.

Method - A flat bottomed dish of not less than 5 cm. diameter is dried in a desiccator and weighed.

3-5 cc. of milk are pipetted into the dish and the latter quickly weighed again.

The difference in these weights gives the weight of milk added.

Heat the dish on a hot water bath till it appears dry. (This process should take 2-3 hours).

Wipe the dish and place in hot air oven at 98-100°C. for an hour.

Cool in desiccator. (Ten minutes if only one dish but longer if more than one hot dish is placed in the desiccator).

Reweigh.

Weight of total solids equals this weight - weight of dish.

Calculate weight of total solids per cent.

95 cc. milk % and yield 0.02. $\% = \frac{0.02 \times 100}{5} = .40\ \%$.

(The weight of milk taken may be calculated from the specific gravity if its volume is accurately measured, e.g., if specific gravity = 1030 and 3 cc. are taken - weight = $\frac{1030 \times 3}{1000}$

= 3.09 g.

Acidity.*- Milk sugar is broken down by bacteria into lactic acid which makes the milk acid or sour, Acidity is usually a sign of high bacterial content or of age. Milk freshly drawn from a cow is generally neutral or faintly alkaline in reaction.

Determination:- Take 10 or 20 cc. of milk and dilute it with an equal quantity of freshly boiled and cooled water.

Add a few drops of phenol phthalein.

Titrate with 0.1N.NaOH until the solution is permanently pink.

Express the result in terms of degrees of acidity, i.e. as the number of cc. of N. alkali required to neutralise 1 litre of milk.

It should not be greater than 25° acidity.

Fat - The fat may be determined by one of several methods. In all processes the fat is separated from the rest of the milk and estimated. The methods differ in the procedure adopted to facilitate this separation.

* For the determination of ash see page 7.

or Stokes Tube

The Werner Schmidt Process - In this method the milk is heated with HCl acid, which dissolves most of the protein and so facilitates the subsequent extraction of the fat by ether. A specially shaped and graduated tube (the Stoke's tube) is used.

1 Method - Place 10 cc. milk in the Stoke's tube.
Add carefully 10 cc. conc. HCl.
2. Gently boil, with careful shaking, until the fluid turns dark brown in colour.
3. Thoroughly cool tube and contents by placing in running water.
Add ether up to the 50 cc. mark (i.e. 30 cc. ether).
4. Insert cork and thoroughly mix contents by repeated inversion for several minutes, e.g. 5 mins.

5. Allow tube to stand vertically for 5 minutes so that the ether separates as an upper layer. This process can often be hastened by gentle tapping. When complete the fluffy layer of casein which forms at the junction of the two fluid layers is generally a compact mass.

Remove carefully a definite volume, say 20 cc., of the ether layer with a pipette and place in a weighed evaporating dish.

Add more ether to the 50 cc. mark (i.e. 20 cc.) and repeat the extraction process.

Remove another 20 cc. of ethereal layer and add to the ether extract in the evaporating dish.

Again add ether to the 50 cc. mark and repeat the extraction process.

Now carefully remove as much of the separated ethereal layer as possible and add to the evaporating dish.

7. Evaporate off the ether by placing the dish on a hot water bath and removing the ether vapour by suction up an inverted filter funnel attached by rubber tubing to a water pump. Make sure that no flame comes near the ether at any stage in this extraction process.

8 Wipe the outside of the dish and place in a hot air oven at 98° - 100° for 30 minutes.

9 Cool in a desiccator (10 minutes at least) and reweigh.

The difference between the final weight and the original weight of the clean dry dish gives the weight of fat extracted from 10 cc. of milk.

Express the result as a percentage.

Rose-Gottlieb's Process - This method is usually employed for determining the fat content of condensed milk. It may be used for milk if 10 g. be taken in place of 2 - 2.5 g.

(a) Transfer 2 - 2.5 g., accurately weighed, of a well mixed sample of condensed milk (10 g. milk) to a suitable apparatus.
(b) Add 8 cc. of warm water, mix well and cool.
(c) Add 1 cc. conc. NH_4OH (S.G. 0.880) and mix.
(d) Add 10 cc. 95% alcohol and mix.
(e) Add 25 cc. of ether and shake vigorously for 1 min.
(f) Add 25 cc. of petroleum ether (B.P. 40° - 60°C.) and again shake vigorously for 30 seconds.
(g) Allow liquids to stand at least ½ hour until the upper ethereal layer is perfectly clear.

(h) Transfer the ether to a suitable dry flask.

(i) Wash the upper portion of the vessel containing the milk by adding 5 cc. ether and then transferring it to the flask. Repeat this washing.

(j) Add 0.5 cc. alcohol and repeat the extraction with 25 cc. ether (shake 1 min.) and 25 cc. petroleum spirit (shake 30 secs.) as before -[(e) to (i) above].

(k) Repeat the extraction once more.

(l) Cautiously distil the solvents from the flask and dry the residual fat at 98° - 100°C. to constant weight, taking the usual precautions to remove all traces of volatile solvent.

(m) Extract the fat from the flask by repeated washings with petroleum spirit, decanting the latter when the sediment has settled.

(n) Dry flask at 98° - 100°C. and reweigh.

(o) The loss of weight of the flask gives the amount of fat removed from the condensed milk or whole milk originally taken.

The Gerber or Centrifugal Method - This is the method most commonly used in this country for the routine analysis of milks, especially when several milks are examined simultaneously. The separation is effected by strongly acidifying the milk with H_2SO_4, which dissolves the protein, and by adding amyl alcohol, which dissolves the fat and is then separated as an upper layer by centrifugal force.

Reagents - It is important that the sulphuric acid and the amyl alcohol used in this method shall be of definite standards of purity and density. Their densities at 20°C. shall be, the acid - 1.815 and the amyl alcohol 0.810-0.812.

A special tube, known as a butyrometer, is used in this method. This tube has a graduated scale upon its neck, from which scale the percentage of fat may be directly read.

Method. - (a) Using the special pipette provided add 10 cc. of the specified sulphuric acid to the butyrometer, taking care not to wet the neck with acid.

(b) Mix the milk thoroughly and withdraw 11 cc. with a pipette of this capacity and carefully add it to the acid in the butyrometer.

(c) Measure 1 cc. of amyl alcohol into the butyrometer.

(d) Without unduly shaking the butyrometer, close the neck firmly with the rubber stopper.

(e) Shake the butyrometer carefully until the curd is dissolved and no white particles can be seen in the liquid. As great heat is developed in this mixing process it is advisable to wrap the butyrometer in a duster.

(f) Place the butyrometer, after placing some distinguishing mark upon it, rubber stopper downwards in the water bath at 68°C. Keep it there until there are sufficient butyrometers to fill the centrifuge.

(g) Transfer the butyrometers to the centrifuge and spin for 4-5 minutes. The transference should be done expeditiously so that the temperature of the butyrometer contents does not fall considerably.

(h) Place the butyrometer again in the water bath for 2-3 minutes.

(i) Holding the tube vertically, read the length of the fat-alcohol column on the butyrometer scale; this gives the percentage of fat. If necessary adjust the rubber stopper so that the whole of the fat column is on the scale.

(j) Replace the butyrometer in the water bath for another 2-3 minutes and then take a check reading of the percentage of fat as rapidly as possible after removal from the bath.

Modifications of this method, including special forms of the butyrometer, are used for determining the fat content of

(a) skim milk, separated milk, and butter milk,
(b) cream, and
(c) cheese.

Soxhlet

Adam's or the Continuous Ether-Extraction Method - In this method hot ether is allowed continuously to extract the fat from a thin dried film of milk on a strip of fat-free paper. The exposure of the milk in such a film facilitates the extraction of the fat. A special apparatus - the Soxhlet extraction apparatus - is used in this method.

Method - (a) Take a strip of fat-free filter paper.

(b) Carefully spread a measured volume, say 2 cc., of milk over the strip, so that the liquid does not run to the edge.

(c) Suspend the strip freely in the air and allow it to dry. (This is best accomplished by leaving overnight. Leave for an hour or two).

(d) Coil the paper strip and place in a hot air oven at 98° - 100° for 1 hour.

(e) During (c) and (d) dry the flask of the Soxhlet apparatus in a desiccator, weigh it, and connect it with the rest of the apparatus.

(f) Place the dried paper strip in the Soxhlet extractor (between the flask and the condenser) and replace the condenser.

(g) Make sure that the water is flowing through the condenser.

(h) Turn out the flame beneath the water bath, insert a funnel into the open end of the condenser and carefully add ether until there is sufficient to siphon over from the Soxhlet extractor into the flask. Add about 10 cc. more.

(i) Allow the extraction to continue for 3 hours, the ether vaporising in the flask, rising into the Soxhlet apparatus and so into the condenser, where it is cooled and returned as liquid into the Soxhlet extractor. Here it bathes the paper coil containing the dried milk until sufficient has collected to siphon over into the flask, taking fat with it. The ether is again vaporised but the fat remains behind in the flask.

(j) Stop the continuous extraction when a large proportion of the ether is bathing the paper coil. Disconnect the flask and distil off the remainder of the ether by immersing in a bath of hot water, taking care that the ether is delivered into a suitable receptacle for collection.

(k) Place flask in the hot air oven at 100°C. for 1½ hours.

(l) Blow air through whilst still hot, place in desiccator to cool and reweigh. The increase in weight of the flask gives the amount of fat extracted from the weight of milk (volume x specific gravity) originally taken. Express the result as a percentage.

This method is unsuitable for sour or homogenised milk.

Densimetric Method -A rough approximation of the fat and "solids-non-fat" of milk can be obtained by the following formulae from the specific gravity of the milk and that of a filtrate obtained by filtering 200 cc. of milk through paper in 15 minutes.

$$\text{Thus } \% \text{ Fat} = \frac{\text{S.G. whole milk} - \text{S.G. filtrate}}{0.0008}$$

$$\text{and } \% \text{ Solids-non-fat} = \frac{\text{S.G. filtrate} - 1}{0.004}$$

There is a legal standard for the fat content of milk, viz. 3%. Many cows give milk containing well over this value, especially such breeds as Jersey and Guernsey. Owing to the excessive breeding of certain herds for quantity, however, certain cows give normal milk containing less than 3% fat. The milk from such cows should be mixed with that from animals giving milk with high fat content. Bulked milk is unlikely to have a fat value below 3%.

If on examination of a "milk" it is found to contain less than 3% fat then it is usual to state the percentage of "genuine milk", i.e. milk containing 3% fat, present in such an "apparent" milk. Thus an apparent milk containing 2.5% fat would contain

$$\frac{2.5}{3} \times 100 = 83.3\% \text{ of genuine milk.}$$

The amount of water added to a milk is generally calculated from the solids-non-fat percentage. Solids-non-fat = total solids-fat.

There is a legal standard for solids-non-fat, this being 8.5% for whole milk and 8.7% for skimmed milk.

In examining a whole milk, then, it is assumed that no water has been added unless the value for solids-non-fat falls below 8.5%. If the value is less than this, say 7%, then the amount of genuine milk in the sample will be

$$\frac{7}{8.5} \times 100 = 82.3\%$$

$\therefore$ the added water = 100-82.3 = 17.7%

The cryoscopic method for the detection of added water is described later.

Proteins in Milk.

Cow's milk contains about 3% of casein and about 0.4% albumen. Usually only the total protein is determined.

Qualitative Test - The presence of protein in milk can be ascertained by the Biuret test. Make a small quantity of milk alkaline with NaOH and add a drop or two of $CuSO_4$ solution. A violet colour develops.

Quantitative estimation (Kjeldahl process). - In this process the organic matter of the milk is oxidised and the protein nitrogen converted into ammonium sulphate. The ammonia is then liberated by making the solution alkaline and distilling, the ammonia being estimated by receiving it in a known quantity of standard acid. From the excess acid remaining the percentage nitrogen can be calculated and this figure by multiplication by a suitable factor gives the protein content of the milk.

Method - (a) Place a known quantity, e.g. 5 g. or 5 cc., of milk in a Kjeldahl flask.

(b) Add a crystal or two of $CuSO_4$, about 10 g. of K_2SO_4 and 25-30 cc. of nitrogen free conc. H_2SO_4.

(c) Place the flask in an inclined position in a fume cupboard and heat gently until frothing ceases.

(d) Boil briskly until the fluid becomes colourless or pale blue and continue for a time after this. This usually requires 2 hours.

(e) Cool.

(f) Dilute with 200 cc. water and pour into a distillation flask.

(g) Add a few pieces of granulated zinc or pumice stone to facilitate even distillation.
(h) Carefully add down the side sufficient NaOH to make the solution strongly alkaline. This point should be indicated by the light blue colour turning dark blue.
(i) Connect the distillation flask with a condenser and a receiving flask containing a measured quantity (say 25 cc.) of standard acid (0.1N), the latter containing a few drops of methyl red as indicator.
(j) Make sure the delivery tube from the condenser projects just below the surface of the acid. It should not project deeply.
(k) Distil until the distillate is no longer alkaline to litmus.

(l) By titration with standard alkali determine the quantity of unneutralised acid in the receiver and so calculate the amount neutralised by the ammonia.
(m) Express this result as the percentage of N in the milk.
(n) Multiply this figure by the factor 6.38 to obtain the percentage of protein in milk.

A blank experiment should be done upon the reagents used to ensure that these contain no N.

Ash

The ash may be defined as the residue left after gently charring and igniting the total solids until all the carbon is removed, the heating being carried out at a temperature sufficiently low to avoid the volatilisation of chlorides. The ash consists of mineral matter, mainly NaCl, KCl, citrates, phosphates of K, Mg and Ca, and a small amount of Ca that was combined with protein.

Determination - Dry a crucible in a desiccator, weigh it and add 20 cc. milk. Quickly weigh again. Evaporate to dryness, carefully to avoid loss of weight by spluttering. Finally ignite at a temperature below redness till the ash is free from carbon. Cool in desiccator. Weigh. Express the result as percentage weight.

Milk B. Spg 1030.
Total Solids 11.78%
Fat.
Lactose
Weight gorsh. 98.8020

10 cc milk - Spg - 1030
25 cc Benedict Solw requires 16 cc filtrate -
25 cc. Benedict = 16 cc. filtrate = ~~10~~ milk,
16 cc. milk filtrate contain 0.0678 gm lact.
∴ 1 cc milk filtrate = 0.0678X / 16c.
10 cc. whole milk diluted to 100cc = 0.0678X10X10 / 16.
10cc milk = 10.30gm.
i.e. 10.32gm of milk = 0.0678X100 / 16.0
∴ 100gms milk - = 0.0678X100X100 / 16 X 10.30

6180
1030
16480 (16480)

4.12
06 78.0000
65 720
208.000
16480
143 2000
32960
109040

Milk (Cont.)

Determination of Lactose or Milk Sugar - The lactose of milk may be determined by a polarimetric or a copper reduction method. Whichever method is adopted the proteins must be removed by precipitation first.

A. Polarimetric method - This method depends upon the fact that lactose in solution rotates the plane of polarised light to the right. The proteins are first precipitated from the milk by acid mercuric nitrate followed by phosphotungstic acid and the clear filtrate is then examined in the polariscope. From the rotation produced the actual amount of lactose in the filtrate and so in the milk can be calculated.

B. Volumetric determination, using Fehling's or Benedict's Solution.

To 10 ccs. milk in a 100 ccs. volumetric flask add 5 cc.s 5% Acetic acid. Agitate gently and allow to stand for five minutes. Dilute to 100 ccs. Mix and filter. Transfer to a burette.

Take 10 cc. of recently mixed Fehling's solution, add 30 cc. water, and bring it to a boil. Whilst gently boiling run in the milk filtrate until the blue colour is just discharged. [The end point may be determined by filtering a small amount of the copper solution through a very small filter paper without funnel. Allow the filtrate to fall on a white tile, acidify with acetic acid and add one drop of freshly prepared, dilute solution of potassium ferrocyanide. If no brown colour is produced the fehling's has been completely reduced.]

If Benedict's solution is used 25 cc. is taken and 3-4 g. (about 1" deep in a dry test tube) of anhydrous sodium carbonate added.

Heat until most of carbonate is dissolved.

Run the milk filtrate from the burette slowly until a bulky white precipitate is formed and the intensity of the blue colour is greatly lessened.

From this point the lactose solution is added more and more slowly, with constant boiling, until the disappearance of the last trace of blue colour.

Best results are obtained when about 10 cc. of the milk filtrate are required by 25 cc. Benedict's reagent. If less than 5 cc. filtrate is required the filtrate should be suitably diluted and the titration repeated.

The titration should be repeated twice and the mean of the last two figures taken.

Calculation

10 cc. milk of specific gravity 1032 were treated with colloidal iron to remove the proteins.

10 cc. Fehling's solution (or 25 cc. Benedict's solution) required 17.5 cc. of filtrate.

Now 10 cc. Fehling's sln. ≡ 25 cc. Benedict's sln. ≡ 0.0678 g.lactose

∴ 17.5 cc. milk filtrate contain 0.0678 g. lactose

∴ 10 cc. of whole milk Moore filtrate " $\frac{0.0678 \times 100}{17.5}$ g. lactose

i.e. 10.32 g. of milk " $\frac{0.0678 \times 100}{17.5}$ g. lactose

∴ 100 g. milk contain $\frac{0.0678 \times 100 \times 100}{17.5 \times 10.32}$ g.

= 3.75 g. lactose

Vieth's Ratio.

The following is an empirical ratio for the proportions of sugar:protein:ash = 13:9:2

From the determination of one of these constituents one or both of the others may be calculated.

Richmond's Scale is another empirical method for calculating the total solids from the specific gravity and the fat percentage. Thus

$$T.S. = 0.25G + 1.2F + 0.14$$

where G = Sp.gr. milk - 1000

and F = the fat percentage.

Other constituents of milk include small quantities of citric acid, urea, peptones, volatile acids and vitamins A, D and C. No colouring matter or preservative is allowed in milk. A small quantity of dirt may be present but this should not exceed 10 mg. per litre.

Dirt may be estimated by allowing a litre of milk to stand for some hours in a conical flask with the narrow end downwards, or by centrifuging the milk, decanting off the supernatant fluid and then washing the residue several times with water, finally collecting the dirt on a filter paper and weighing it.

Cream. The determination of the percentage of "cream" (not milk fat) in milk may be estimated by allowing 100 cc. of milk to stand in a cream tube (a special graduated cylinder) for 6-10 hours. The volume of the cream layer is then read off from the scale, each graduation representing 1%. The average yield is 8%. Boiled and "homogenised" milk give a very small percentage of cream.

Regulations respecting Milk.

1. The solids-non-fat must not be less than 8.5% in whole milk. Sale of Milk Regulations, 1901.

2. The milk-fat must not be less than 3%. Ibid.

3. No preservative is allowed in milk. Here the word "milk" includes skimmed, condensed and dried milk. Milk and Cream Regulations, 1912.

4. In separated or skimmed milk the solids-non-fat must not be less than 8.7%. Sale of Milk Regulations, 1912.

5. No colouring matter is allowed in milk or cream. Milk and Dairies (Amendment) Act, 1922.

The Analysis of Milk for the Purpose of Detecting Adulteration.

The commonest forms of adulteration which the analyst may have to detect and to make quantitative estimations of when possible are:

1. The addition of water.
2. The abstraction of fat.
3. The addition of separated milk.
4. " " " condensed milk, boiled milk, or sterilised milk.
5. " " " gelatin.
6. " " " starch, dextrin, cane sugar, glucose, or invert sugar.
7. " " " preservatives. } Prohibited by law.
8. " " " colouring matters.

Added Water and Abstraction of Fat (1) and (2).

Provided the milk is fresh and in good condition, the analyst should determine (a) specific gravity, (b) fat, (c) total solids, (d) solids-non-fat. Frequently the total solids figure is calculated from Richmond's scale and the specific gravity and fat determinations. Then this figure less the fat gives the figure S for solids-non-fat. Then % added water = $100 - \frac{S \times 100}{8.5}$

as 8.5 is the legal minimum for solids-non-fat.

N.B. The specific gravity is lowered by the addition of water and raised by the abstraction of fat.

For the detection of added water most analysts now use the cryoscopic method. This method depends upon the fact that the freezing point of a normal cow's milk is approximately -0.550°C., i.e. it is 0.550°C. lower than the freezing point of water. This lowering of the f.p. of the water of the milk is due to the presence of the substances (salts) dissolved in it. When the milk is diluted with water the concentration of these substances is lowered and hence the f.p. depression is less. The f.p. now lies between -0.550°C. and 0°C. If the f.p. of a watered milk is accurately determined the amount of water added can be calculated from the following formula

$$W = \frac{100\,(T - T')}{T}$$

Where W = percentage of added water
T = the average depression of the f.p. due to a normal milk (i.e. 0.550°C.)
and T' = the depression of the f.p. due to the watered milk.

For this determination a special apparatus-called a Hortvet cryoscope - is used. In this apparatus about 30-35 cc. of milk can be carefully frozen and the temperature of freezing noted on a thermometer reading to 0.001°C. This cryoscopic method is useless with sour milk.

(3) Adulteration with separated milk - The adulteration of whole milk with separated milk, i.e. milk from which fat has been abstracted, was fairly common, especially in large towns. It can only be detected when the original composition of the milk (as supplied by the farmers) is known or when the addition is excessive and the fat value of the adulterated milk falls below 3%.

(4) The adulteration of fresh milk with unsweetened condensed milk, boiled milk or sterilised milk.

This can be detected by a lowered albumen figure, for heating milk at 75°C. for 10 minutes reduces the % albumen from 0.4 to 0.07,

whilst heating at 80°C. for 10 minutes removes all the albumen.

A boiled or pasteurised milk may be detected by the fact that heating a milk destroys an enzyme, called a peroxidase. The test for this enzyme is as follows: Treat 5 cc. of milk in a test tube with 2 drops of a 2% solution of p.phenylenediamine, and add 1 drop of a very dilute solution of H_2O_2. (A 10 vol. solution diluted 1 in 50.) If the milk is fresh a dark bluish violet colour develops immediately. If the milk is pasteurised a weaker colour may develop slowly and if the milk has been boiled or sterilised no colour will develop.

(5) and (6) Artificial thickening of cream. Gelatin, starch, dextrin, cane sugar, glucose, invert sugar, etc. have been used as thickeners of cream.

To test for gelatin, 10 cc. of water are added to 5 cc. of cream and then 1 cc. of acid mercuric nitrate solution to precipitate proteins and remove fat. Shake well and filter. To the filtrate a saturated solution of picric acid is added and a yellow precipitate is produced in the presence of gelatin.

Starch and dextrin may be detected by their reactions with iodine (starch → blue, and dextrin → reddish brown).

Phosphatase Test

Milk contains an enzyme called phosphatase, which converts some of the organic phosphorus present into inorganic phosphorus. By a simple colorimetric test the amount of inorganic phosphorus produced under standard conditions can be measured and so an estimate of the quantity and activity of the enzyme can be determined. This test is used to ascertain whether milk has been efficiently pasteurised as the enzyme is destroyed when pasteurisation by the holder process has been carried out accurately.

(7) Preservatives. No preservative of any kind is now allowed in milk or milk products. The commonest preservatives added to milk are boric acid or borates, formaldehyde, salicylic acid, or hydrogen peroxide. Suitable tests for these substances are given below.

Boric acid, borates

(a) Preliminary test - Immerse a strip of turmeric paper in a sample of the milk acidified with HCl. (Add 1 cc. conc. HCl to 10 cc. milk.) Allow the paper to dry in the air. If borax or boric acid is present the paper turns a chafacteristic red colour, which is changed to a dark blue-green by ammonia. The red colour is restored by acid.

(b) Confirmatory test - Make about 25 cc. of milk decidedly alkaline with lime water and evaporate to dryness on a water bath. Ignite the residue in a crucible at a low red heat till all the organic matter is thoroughly charred. Cool, add about 15 cc. of water and thoroughly mix. Add HCl drop by drop until the solution is distinctly acid. Saturate a piece of turmeric paper with the solution and allow it to dry spontaneously. If borates are present the red colour described above is obtained.

(c) Glyceroborate test - This test depends upon the fact that borates combine with glycerine forming glyceroborates which are less strongly ionised and therefore less alkaline than sodium borate.

To 5 cc. milk add 3 drops phenol phthalein and make just alkaline with caustic soda, i.e. till pink colour made permanent.

Similarly take 5 cc. of 50% glycerine and make just alkaline to phenol phthalein.

Mix the adjusted milk and the 50% glycerine. If boric acid or borates are present the pink colour is discharged.

(a) Leach test - Mix in a porcelain dish about 10 cc. milk with an equal volume of HCl-$FeCl_3$ solution (i.e. 500 cc. of HCl containing 1 cc. of 10% ferric chloride).

Heat slowly to 80-90° directly over a gas flame, rotating dish to break up curd.

A violet colour is developed if HCHO is present..

(b) Hehner's test - To about 6 cc. milk in a wide test tube carefully add about 3 cc. commercial H_2SO_4 so that an acid layer forms at the bottom. If HCHO is present a violet or blue ring is obtained. [This test is given only in the presence of a trace of iron or other oxidising agent.]

"Mystin" is a preparation containing a mixture of formaldehyde (about 0.3%) with sodium nitrite (10%). The presence of the latter masks the usual tests for formaldehyde. The nitrite may be detected by adding metaphenylenediamine solution (diluted 1 in 5) to about 10 cc. of milk in a test tube containing a few drops of dilute HCl. Mix and allow to stand, when after some time a yellowish orange colour will develop if nitrites are present. If difficulties arise owing to the colour of the reagent a control can be put up with normal milk.

The nitrite may be removed by treatment with urea and sulphuric acid and the milk then tested for the presence of HCHO.

To 5 cc. of a milk containing HCHO and nitrite add 2.5 cc. of 2% urea solution and 1 drop of dil. H_2SO_4. Heat in a boiling water bath for about 2 minutes, cool, and add commercial H_2SO_4. A purple ring at the junction of the fluids indicates "mystin".

Salicylic Acid

(a) Acidify 100 cc. of the milk with 5 cc. HCl (1-3), shake until curdled and filter.

Add 50-100 cc. of ether to the precipitate and shake well.
Remove ether to porcelain dish and evaporate off most of ether on a hot water bath taking the usual precautions.
Allow the last few drops of ether to evaporate spontaneously and then add a drop of 0.5% neutral ferric chloride solution to part of the residue.
If salicylic acid is present a violet colour develops.

(b) Extract the acidified milk with ether as above.
Dissolve part of the residue in a little hot water.

Cool 10 cc. in a test tube.

Add 4-5 drops of 10% potassium nitrite, 4-5 drops of 50% acetic acid and 1 drop of 1% copper sulphate solution. Mix thoroughly.

Heat to boiling. Boil for half-a-minute and stand 2 minutes. If salicylic acid is present a Bordeaux red colour develops

Hydrogen Peroxide may be detected in unboiled milk by the peroxidase test already referred to. If the milk is boiled then some unboiled milk known not to contain peroxide can be added.

Take 5 cc. of milk, add 2 drops of 2% p.phonylenediamine solution.

If H_2O_2 is present a bluish violet colour develops.

(8) Colouring Matters. The colour of an unadulterated milk is due to the presence of carotene, the precursor of vitamin A.

Colouring matter is sometimes added to milk to make it look richer, although this addition is illegal. The colouring matters most commonly used are annatto or one of the coal tar dyes. They may be tested for as follows.

(a) Annatto. Warm about 150 cc. milk in a covered dish over a flame and add about 5 cc. acetic acid (1-3). Slowly continue heating nearly to the boiling point whilst stirring.

Collect the curd if possible on the stirring rod, (if not filter) and pour off the whey.

Press curd free from liquid and transfer to a small flask.

Macerate and allow to stand several hours (preferably overnight) in about 50 cc. ether, keeping flask tightly corked and shaking at intervals.

Decant off bulk of ether and evaporate off remainder.

Make residue alkaline with NaOH and pour on to a small wet filter paper.

If annatto is present it is adsorbed by the wet filter paper, which becomes straw yellow. The colour is retained on washing with water.

Dry filter paper and add a drop of stannous chloride solution.

A pink colour is developed if annatto is present.

(b) Coal Tar dyes. The curd of an uncoloured milk is perfectly white after complete extraction with ether. So also is the curd of milk coloured with annatto. If, after extraction, as above, with ether the curd is orange or yellow, then a coal tar dye is probably present.

On acidification of a milk with HCl a pink colour is developed when a coal tar dye is present.

Cream.

The following figures indicate the composition of a good (average) sample of cream.

Fat 54.38%, Proteins 4.05%, Ash 0.49%, and Water 40.87%.

It is usually examined by the methods described under milk after the cream has been suitably diluted but its fats are examined by the methods described under butter.

Skim Milk, Separated Milk. The first is obtained by hand and the second by the centrifuge, the latter removing much more of the fat. Skim milk usually contains from 0.8 to 1% of fat and separated milk only from 0.1 to 0.5%.

Study the carefully

The Chief Influences which tend to modify the Composition of Cow's Milk.

Cow's milk is not a product of fixed chemical composition; it may vary considerably as the result of certain influencing factors. These include (1) the bread, (2) period of lactation, (3) conditions as to milking (morning and evening milking; fore milk, strippings, etc.), (4) season of year, (5) climate, (6) land, (7) feeding, (8) health of cow. Naturally the results of these conditions are more marked in the milk of individual cows than with the average mixed milk from a large herd.

(1) Breed. As a rule the richer the quality the less the quantity of milk. Thus Jersey cows give a small quantity of rich milk (fat 5%+) and Short-horns or Friesians a large quantity of medium milk. This explains why farmers often keep one or two Channel Island cows in a herd of cattle of other breed.

(2) Period of Lactation. A cow after calving first yields 'colostrum' but this is not often met with in milk as for the first 4 days after calving the milk from a cow is usually not for sale. The milk for the remainder of the first week is rich in fat, the quality then drops rapidly to a minimum during the second week, after which the quality gradually improves until about a week before drying up, when it rapidly diminishes both in quantity and quality.

(3) Conditions as to milking. When cows are milked 3 times a day the quality is usually the same but with only two milkings, morning and evening, the latter contains as a rule from 0.2 to 0.4% more fat than the former. During a single milking the first portion or "fore" milk contains little fat whilst the last portions or "strippings" contain an excess of milk fat. 1 2 3 4 strippings

0.9 2.6 5.3 9.8

(4) Seasonal Variations are really due to the period of calving and the variability of the food with the time of the year. Generally speaking in November, December and January, milk is rich in fats and solids-non-fat; in February, March and April, somewhat poorer in fats and about the same in solids-non-fat; in May, June, July and August the fat and the solids-non-fat are low and both of these constituents improve in September and October. - Richer in fat in winter than summer

(5) Climate. An escessively dry summer is liable to yield milk of low solids-non-fat content whilst an excessively wet season usually means a somewhat poorer or watered milk. The amount of sun will lead to a variation in the vitamin content of the milk, especially with vitamin D, which is practically absent from milk in winter.

(6) The type of land upon which a cow grazes will influence its milk indirectly by affecting the composition of the grass and water.

(7) Feeding. It is obvious that a cow feeding daily upon a well-balanced and highly nutritious diet will yield much better and probably more milk than an animal kept on an ill-balanced or poor diet. The type of pasture land will influence the milk inasmuch as it will influence the quality of the grass. Feeding increases more greatly than yield

(8) Disease. Certain diseases, especially local diseases of the udder, will affect the chemical composition of milk but in many other diseases this composition is unaffected although in all cases of definitely unhealthy cows the quantity of milk is diminished.

Age of Cow after 2 yrs for fat content to become lower. Cow can produce up 13 years - Average life be five or six -

Condensed Milk, Butter.

Condensed Milk is prepared by concentrating whole or separated milk, usually to about one third its volume. In order to guard against the production of "boiled" milk flavour the evaporation is carried out at reduced pressure at a low temperature, e.g. about 50°C. The product is not sterile. Cane sugar may be added both to impart a sweet taste and to act as a preservative.

The sale and standards of condensed milks are governed by the Public Health (Condensed Milk) Regulations, 1923. Four types of condensed milk are allowed and they must contain the following minimum amounts of fat and total solids.

include F. S. P. salts + Sucrose

(1)	Full cream, unsweetened	9.0%	Milk Fat	31% Total Solids
(2)	" " sweetened	9.0%	" "	31% " "
(3)	Skimmed, unsweetened	-		20% " "
(4)	" sweetened	-		26% " "

For analysis, condensed milk is usually diluted 1/5 and analysed as for milk. The fat is generally estimated by the Rose-Gottlieb process and the lactose and sucrose may be determined by copper reduction or polarimetric methods.

Determination of Lactose. Take 5 cc. of the diluted condensed milk (1 in 5) in 100 cc. flask.

Add a few drops of mercuric nitrate solution and make up to 100 cc. with distilled water.

Filter, add ammonia to the filtrate till alkaline.

Estimate the amount of lactose by Fehling's or Benedict's method.

Cane Sugar, Sucrose.

Both sucrose and lactose are disaccharides, i.e. they are compounds of two simple sugars. By suitable treatment these sugars can be split up into their components. Thus sucrose is hydrolysed into glucose and fructose whilst lactose breaks down into glucose and galactose. All these simple sugars have the power of reducing Fehling's solution but of the disaccharides only lactose will reduce copper solutions, sucrose being a non-reducing sugar. Storing mineral acids of suitable concentration will hydrolyse both sucrose and lactose but a weak organic acid, e.g. citric acid, under certain conditions will hydrolyse sucrose but not lactose. After treating with citric acid therefore a sucrose solution will reduce Fehling's because of the glucose and fructose formed. If then we have a solution containing both sucrose and lactose and this be treated with Fehling's solution then the amount of reduction that takes place will be a measure of the lactose only (as the sucrose does not reduce Fehling's). If this solution of sucrose and lactose be boiled with a 2% solution of citric acid then the lactose is untouched whilst the sucrose will be broken down into fructose and glucose. The solution will now be more strongly reducing because in addition to the reduction due to the lactose there is that due to the glucose and fructose obtained from the sucrose. By determining the reducing power of the solution before and after hydrolysis with citric acid therefore the amounts of lactose and of sucrose can be estimated.

Calculation. To determine the amount of sucrose and lactose present in a solution containing both.

By experiment it is known that

10 cc. Fehling's (or 25 cc. Benedict's) soln. ≡ 0.0678 g. lactose

or ≡ 0.0475 g. sucrose (after hydrolysis)

Suppose 10 cc. Fehling's was reduced by 15 cc. of solution

Then, as the reduction is entirely due to lactose,

15 cc. of solution contain 0.0678 g. lactose

100 cc. " " " 0.452 g. "

But after hydrolysis with citric acid, it is found that

10 cc. Fehling's is reduced by 5 cc. of hydrolysed solution

If this reduction was brought about only by the hydrolysed sucrose, then

5 cc. of solution would contain 0.0475 g. sucrose

∴ 100 cc. " " " " 0.95 g. "

But part of this reduction is actually due to lactose and therefore this figure for sucrose should be decreased by the equivalent of the lactose present.

From the 1st titration we know 100 cc. of solution contain 0.452 g. lactose

Now 0.0678 g. lactose ≡ 0.0475 g. sucrose

∴ 0.452 g. " ≡ $\frac{0.0475 \times 0.452}{0.0678}$ g. sucrose

= 0.317 g. sucrose

∴ 100 cc. solution actually contain 0.95 - 0.317 = 0.633 g. sucrose

This method of estimating sucrose in the presence of lactose is used to determine the amount of cane sugar that has been added to a condensed milk.

Method- After determining the lactose by a direct reduction estimation determine the sucrose as follows:

Add a drop or 2 of strong HNO_3 to 5 cc. of a 1/5 condensed milk.

Allow the mixture to stand unshaken for 5 minutes, then add water cautiously to 50 cc.

Filter, wash filter paper twice with 10 cc. water.

Add about 1.5 g. citric acid to the total filtrate in a conical flask and bring to a boil for 40 minutes, with a filter funnel in the neck to act as a condenser.

Keep the volume approximately constant.

Add ammonia until alkaline and make the volume up to 100 cc. and place in a burette.

Take 10 cc. Fehling's (or 25 cc. Benedict's) solution and titrate it with the hydrolysed milk filtrate.

From this titration and the previous one with the unhydrolysed milk filtrate calculate the amount of sucrose present in the milk.

Qualitative Tests for Sucrose.

(1) Add 2 cc. of ammonium molybdate and 8 cc. of HCl (1:8) to 10 cc. of the diluted milk. Heat below 80°C. for 5 minutes. A blue colour develops if sucrose is present.

(2) To 15 cc. of the diluted milk add 1 cc. of HCl and 0.1 gm of resorcinol. Boil. A red colour denotes sucrose.

Dried Milk may be sold subject to grading defined by the Public Health (Dried Milk) Regulations, 1922 and 1927. The grades must contain not less than the following % of milk fat.

Dried	full	cream	milk	26% Fat
"	3/4	"	"	20% "
"	1/2	"	"	14% "
"	1/4	"	"	8% "

Dried milk is usually manufactured by one of two methods. In the roller method the milk is allowed to fall between two horizontal revolving cylinders almost touching each other. The rollers are heated internally by compressed steam and therefore have a temperature above 100°C. and so tha water rapidly evaporates from the milk. The dried milk is mechanically scraped off the rollers and collected. In the spray method pasteurised condensed milk is forced by means of a jet of hot air through a small orifice into a large heated (over 116°) chamber. The milk spray rapidly loses its water and the powder falls to the ground.

More vitamin C is said to be retained by the roller than by the spray method.

Analysis. - The methods of analysis are similar to those for milk except that the powder must first be obtained in solution. This can usually be done by shaking hard with warm water. It is sometimes facilitated by the addition of a little ammonia. The fat estimation is best carried out by the Werner Schmidt method.

Dried milk is often a constituent of baby foods, mixed with other products. The presence of starch may be detected by the iodine test and sucrose can be detected by either of the tests given above.

Koumiss is an alcoholic drink made by the fermentation of milk (mare, camel or cow).

Butter milk is the thin whey left behind when the fat has been extracted in the process of butter making. It is essentially a dilute, poor acid milk. An average composition is:

Water 90.62%, casein 3.78, fat 1.25, lactose 3.38, lactic acid 0.32 and ash 0.65

Butter

Butter is prepared by violently agitating cream in a suitable apparatus until the fat globules coalesce, entangling some casein and serum; the butter is well pressed to free it from moisture as much as possible and salt is added to assist its preservation. Butter, therefore, is principally composed of milk fat with a small and a variable quantity of water, casein, milk sugar and ash, the latter consisting chiefly, but not entirely, of the salt added. An average butter consists of fat 83.5%, casein 1.0, sugar 1.0, ash 1.5 and water 13. Certain legal standards are laid down for butter and margarine in the Sale of Butter Regulations, 1907, viz.

(1) The legal maximum for water in butter is 16%. but it may be up to 24% in "milk blended butter".

(2) The legal maximum for water in margarine is 16%.

(3) Margarine must not contain more than 10% butter fat and it must not be sold under any title suggestive of butter.

Analysis and Adulteration of Butter. The only common adulterations of butter are:

(1) The substitution or admixture of fats other than butter.
(2) Excess water, either fraudulently added or left in by faulty manufacture.
(3) The addition of colouring matters.
(4) The addition of boric acid or other preservative.

The analysis of butter may be a general one, estimating the amount of water, fat, etc. present or it may be an examination into the actual character of the fat. The latter, except examination for preservatives, is the more important to the Public Health Officer.

Microscopically ordinarily well-made butter will have a characteristic appearance, the water globules varying in size within narrow limits. Margarine appears quite different, the ground substances having a coarsely granular appearance with some very large water globules.

The Proximate Analysis of Butter, i.e. its separation into mineral matter (ash), curd, butter fat and water, is rapidly performed.

Care should be taken to secure an adequate sample. About 8 oz. should be taken, usually from four vertical borings made into separate parts of the box containing the butter. The separate borings should be placed into a wide mouth bottle, with certain precautions, and before analysis heated in a steam oven with frequent shaking till all is melted. It is then allowed to cool with constant shaking so that all the samples are well mixed, and portions are taken for analysis at once whilst the butter is still in a pasty mass.

(1) Water Content. - Weigh a dried porcelain dish of 3 inch diameter and 1 inch deep and preferably with a flat bottom, together with a glass stirring rod.

(2) Add a sample of butter about 5-6 g. and weigh accurately.
(3) Heat on a boiling water bath with frequent stirring until the water has evaporated and the curd is dry.
(4) Wipe off the water on the outside of the dish and transfer for 30 minutes to a hot air oven at 98° - 100°C. [Do not place on the bottom of the oven unless there is a shelf allowing circulation of air below].
Cool in a dessicator and weigh.
The loss in weight gives the weight of water in the amount of butter taken. Express the result as a percentage.

Important.

Curd and Salt. - The curd and salt may be estimated on the same sample by melting the fat and then adding about 30 cc. of petroleum spirit (b.p. 40° - 60°C.), mixing and macerating the sediment with the glass rod. Allow to settle and then carefully pour off the ether fat layer taking care not to lose any sediment.
Repeat this extraction at least twice with 20 cc. petroleum ether, until no fat remains, then evaporate off the ether, dry the dish and weigh.
The weight of the curd and salt will be given by the difference between this weight and the weight of the clean, dry dish.