ESTIMATION OF SOLIDS-NOT-FAT IN MILK USING NEW SPECIFIC GRAVITY LACTOMETER AT 27° C

A DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT
OF THE REQUIREMENTS
FOR
THE DEGREE OF

MASTER OF SCIENCE

IN

DAIRYING

(DAIRY CHEMISTRY)
TO
THE KURUKSHETRA UNIVERSITY
KURUKSHETRA

By
MUKESH DUTT SHARMA

DIVISION OF DAIRY CHEMISTRY
NATIONAL DAIRY RESEARCH INSTITUTE
(I.C.A.R.)
KARNAL (HARYANA)
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TO

MY PARENTS

Dr. B.S. Bector, M.Sc.(Dairying),Ph.D.
Scientist S-1 (Dairy Chemistry)

DIVISION OF DAIRY CHEMISTRY
NATIONAL DAIRY RESEARCH INSTITUTE
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Dated ~~the~~ 12th February, 1982

This is to certify that Shri Mukesh Dutt Sharma of the National Dairy Research Institute, Karnal, participated in the planning of this study, carried out the experimental work involved, analysed the data and prepared this report on 'Estimation of Solids-Not-Fat in Milk Using New Specific Gravity Lactometer at 27°C'. He did this in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in Dairying (Dairy Chemistry) of Kurukshetra University, under my supervision. Help and assistance given by individuals as well as Institutions in the execution of the work has been suitably acknowledged.

(B.S. BECTOR)

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Lastly, but not the least, I place on record my respects to my parents for their encouragement throughout my studies at the Institute.

Mukesh Dutt.

(MUKESH DUTT SHARMA)

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CHAPTER I

INTRODUCTION

INTRODUCTION

The chemical quality of milk is assessed by its fat and solids-not-fat (SNF) contents. It is, therefore, very important to estimate the fat and the solids-not-fat content of milk correctly and speedily, especially for milk delivered at collection centres where milk is paid for according to its fat and solids-not-fat content. A variety of methods such as gravimetric, volumetric and instrumental, etc. have been proposed and widely employed for the determination of fat and solids-not-fat contents. However, none of them satisfies the requirements for both speed and precision. The gravimetric methods are more accurate but they are more time-consuming and demand for better analytical skill. At field level, the volumetric methods are more suitable and widely used since a large number of samples are to be tested within a short time. Among the various volumetric methods of estimation of fat, the Gerber method is quick and convenient and is mostly followed in our country.

Several lactometric procedures have been proposed by different workers for the estimation of total solids/ solids-not-fat in milk. These methods are rapid and simple. For rapid and routine estimation of SNF (solids-not-fat) under field conditions, there is no better method than using a hydrometer or a lactometer and then using a formula

to calculate solids-not-fat from the hydrometer or lactometer reading and the fat content of milk.

In 1965, Indian Standards Institution prescribed specifications for two types of hydrometers for use in milk for solids-not-fat determination by calculations (IS:1183-1965). These specifications were adopted from the British Standards Institution. Unfortunately, the hydrometers of these specifications are not available in our country. Keeping in view the non-availability of properly calibrated hydrometers and the preference of users to use specific gravity lactometers only, as was revealed through data collected from various dairies in the country, ISI has prescribed specifications for a new specific gravity lactometer calibrated at 27°C in its standard IS: ~~9585-1980~~ 1183-1981. A formula for calculating solids-not-fat using this new lactometer has also been suggested, which is being tested at different dairy centres, as part of field trials.

A systematic study, therefore, has been conducted to examine the suitability of this lactometer as well as the new formula for the determination of solids-not-fat in milk.

CHAPTER II

SCOPE AND PLAN OF WORK

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Recently, a new specific gravity lactometer calibrated at 27°C has been recommended by Indian Standards Institution in its standard IS: 1585-1980 for the determination of solids-not-fat (SNF) in milk by the volumetric method. In this standard, a new formula (SNF = 0.25 L + 0.25 F + 0.60) has also been proposed when the above-mentioned lactometer is used for the calculation of SNF values. This formula is being tested at different dairy centres as part of the field trials.

Therefore, it was proposed to examine the suitability of this lactometer as well as the new formula for the determination of SNF value in milk. From the results obtained, the new formula will be suitably modified or changed so that the variations in the SNF values are minimised.

Milk samples (individual cow and buffalo milk and pooled) will be analysed for the following:

1. Total solids by gravimetric method.
2. Fat will be determined by Gerber method using 10.75 ml milk pipette.
3. Total solids/SNF will be calculated by following the new formula -

   TS = 0.25 L + 1.25 F + 0.60

   or SNF = 0.25 L + 0.25 F + 0.60

   where, L = lactometer reading at 27°C

   F = percentage of fat in milk.

The results obtained will be statistically analysed to see the extent of variation and the formula will be suitably modified or changed, if necessary, so that the SNF values obtained by the new formula are in close agreement with the gravimetric values.

CHAPTER III

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Since the precise and speedy estimation of milk fat and solids-not-fat is important, a variety of methods for their determination have been suggested from time to time. These can be divided into the following three classes:

1. Gravimetric methods
2. Volumetric methods
3. Instrumental methods

The gravimetric methods though accurate are time consuming, expensive and require costly equipment and skilled personnel. At the field level, volumetric methods are mostly followed. These being comparatively faster, cheaper and simpler for routine analysis purposes. The instrumental methods are very quick and a large number of samples can be routinely analysed with ease, in a very short time span.

Methods for estimation of milk fat :

I. Gravimetric methods :

These are considered to be having highest precision and the other rapid methods are compared and standardized against them. The basic principle being the extraction of milk fat from a known weight of milk, with a suitable solvent(s) which is/are subsequently evaporated.

Rose-Gottlieb method is preferred among all other gravimetric methods, because in this method, the milk fat is not subjected to drastic pre-treatments with acid or by heating and also the process is fairly rapid and gives very clear separation of fat solvents from the aqueous layer. Rose (1888) gave the principle of fat extraction from milk using ammonia and ethyl alcohol, while Gottlieb (1892) made certain improvements to this method. He added, to 10 ml of milk, 1 ml of conc. ammonia, 10 ml of ethyl alcohol, 25 ml of diethyl ether and 25 ml of light petroleum, shaking the mixture after each addition. The flask is then kept undisturbed for 30 min to facilitate the separation of ethereal layer from aqueous layer. The ethereal layer is then siphoned off into a tared flask and the extraction is repeated twice with 15 ml each of diethyl ether and petroleum ether. After combining all these separations, the ether is evaporated off in a boiling water bath. From the weight of the residue obtained, the percentage of fat is calculated. This method is universally accepted as a standard for comparing the accuracy of other methods.

Principle of Mojonnier method is same as that of Rose-Gottlieb method, but the shape of the Mojonnier tube is convenient for easy decantation of the solvent.

Adams (1885) adopted a method consisting of soaking a known amount of milk on the 2.5" X 22" filter paper strip and drying in an oven at 100°C. It is then transferred to

the Soxhlet extractor and the fat extracted by ether which is then evaporated. From the weight of the residue, fat percentage is then calculated.

Mulder and Myer's (1953) method consists of boiling 6 ml of conc. HCl with 8 g of milk in a Mojonnier tube. Then 8 ml of ethyl alcohol is added and extraction is repeated 3 times with petroleum ether.

Mulyarchuk and Andreevskaya (1955) described one method in which to 5 ml of milk, 5 g of calcinated soda is added which is extracted with 20 ml solvent (benzene or dichloroethane). After crystallization of soda, 10 ml of clear solution is freed of solvent and the milk fat is weighed. The average results were 0.03 to 0.06% higher than the results obtained by Gerber method.

Walstra and Mulder (1962) gave a method based upon extraction of the liberated fat, using slightly modified Mojonnier tube and also explained the importance of the position of the interval of the two phases. The best condition for the evaporation of the solvents and the drying of the fat was also given.

II. Volumetric Methods :

Volumetric methods, which are much faster, cheaper and simpler than the gravimetric methods, are employed extensively in the dairy industry. These are generally based on the

principle that the solids-not-fat in the sample are dissolved or digested, the fat emulsion destroyed by the addition of one or more chemicals and the fat volume measured in a narrow graduated tube. The separation of milk fat is generally promoted by centrifugal force with or without the previous addition of a surfectant. The most remarkable advancement in this field was the development of Babcock and Gerber methods. These methods, even to this date, are extensively used in USA and Europe, respectively. In India Gerber method is used as a simple and quick method for estimating milk fat.

Merchand (1854) developed the first volumetric method of milk fat estimation. He took 10 ml of milk, 1 or 2 drops of sodium hydroxide, 10 ml of diethyl ether and 10 ml of ethyl alcohol. These contents were carefully mixed into a tube called lactobutyrometer. The tube was then placed vertically in a water-bath at 40°C until it cooled to 30°C. When the fat layer had fully separated, the degrees of fat were read from the graduations of the tube and converted to weight of fat in grams. Schmidt (1878) modified Merchand's method by enlarging the lactobutyrometer, so as to facilitate mixing of milk and reagents. He used 3 to 5 drops of 5% acetic acid instead of sodium hydroxide, stronger alcohol and read the fat volume at 20°C instead of at 30°C. Liebermann (1883) contributed a combination of volumetric and gravimetric procedures. Fifty ml of milk was added in a large cylinder

and 5 ml of sodium hydroxide and 50 ml of diethyl ether were added. The contents were mixed and allowed to settle for 10 to 15 min, then a 20 ml aliquot was evaporated in a dry water bath at 40-50°C and dried for 15 min at 100-105°C. The air-free fat was measured in a calibrated flask and converted to per-cent by weight. Delaval (1885) patented a method called the lactometric test for estimating the fat content of milk. He was the first to use a centrifuge.

Test developed by Babcock (1890) consists in measuring 17.6 ml of milk by means of a pipette into a milk bottle, adding an equal amount of sulphuric acid (specific gravity 1.82 to 1.83) and the contents are thoroughly mixed, until the curd has disappeared. It is then centrifuged for five minutes. Sufficient water is then added to bring the contents to the base of the bottle neck. Bottle is again centrifuged for one minute to gather the fat into a coherent column. Test bottles are placed in water bath at 135-140°F and the space filled by the fat column is measured by means of a pair of dividers. This method was compared with Adam's method on 29 samples. The results showed that this test averaged 0.0193% higher with 19 of the 29 comparisons agreeing within 0.10%.

Gerber (1892) gave a method which is easy to perform and in which the separation of fat is further facilitated by the addition of amyl alcohol. He also devised an 11 ml milk pipette on mathematical grounds.

Kothavalla *et al*. (1944) obtained normal Gerber tests without centrifuging by allowing the mixture of milk, sulphuric acid and amyl alcohol to stand at room temperature for 8 hr, and then immersing the tube in a water bath at 160°F, 15 min before reading. By increasing the time in the water bath at 160°F to 20 min, satisfactory results were obtained after 3 hr at room temperature. By increasing the temperature of water bath to 180, 190 and 195°F and immersing the tubes for 20 min the times of standing at room temperature necessary for satisfactory results were reduced to 2, 1.5 and 1 hr respectively They suggested that these modifications could make possible, the regular use of Gerber test by small milk producers. Peltova and Suhonev (1957) modified the above technique by placing the butyrometer in a water bath at 45°C for 1.5 hr followed by 30 min at 65°C. These tests gave clear readings and were in good agreement with the results of the Gerber method.

Radema and Mulder (1948) stressed the fact that the Gerber test is empirical and found that it agreed with the Rose-Gottlieb method according to the expression:

$$G = 1.04\ R.G. - 0.07.$$

From a series of mathematical calculations, Fort (1949) showed the tendency of the Gerber test to give increasingly higher values than the Rose-Gottlieb method. He found that the Gerber scale did not indicate the weight of fat in 100 g but in 100 ml of milk sample and thus confirmed the findings of Chaineux and Simonart (1938).

Labuschagne and Vogt (1960) arrived at the following regression equations from statistical analysis of data obtained from 413 samples of milk: G = 1.041 R.G. - 0.058. Statistical agreement between the results of the two methods over wide range of fat content was found when a pipette which delivered 10.73959 g of water at 20°C was used in the Gerber test. Mishra (1963) used the Gerber test and readings were read after putting the butyrometer in water bath at 60°C/20 min. He found that the modified Rose-Gottlieb method was related to Gerber reading (G) by the regression equation:

$$G = 1.05 \text{ R.G.} + 0.27$$

Bolotov (1960) developed a technique in which milk is centrifuged at 1500-2000rpm/10-20 min and a drop of methylene blue is added to produce a sharper division between fat and skim milk. The maximum deviation of the results from those by Gerber method were $\pm$ 0.30%.

Konard and Zuhlsdorf (1962) recommended the use of 10.75 ml pipette in the Gerber method over the 11 ml pipette. The results obtained were compared with the Rose-Gottlieb method and it was indicated that this pipette was preferable over 11 ml pipette for products with commonly occurring fat contents.

The suitability of Gerber test against the standard gravimetric Rose-Gottlieb method was examined by Pruthi and Bhalerao (1968). They observed that with increasing fat in

milk, Gerber test gave increasingly higher values than the gravimetric method. They calculated 10.75 ml milk pipette from the experimental data which, when used for Gerber test for buffalo milk, gave close readings with Rose-Gottlieb method. Subsequently, Ghouse and Jain (1973) confirmed their findings and proposed 10.75 ml milk pipette for the Gerber method under Indian conditions. On the basis of these suggestions, Indian Standards Institution decided to replace 11.04 ml pipette by 10.75 ml milk pipette in IS : 1223(Part II)-1972 which is now used by most of the dairies in India.

Pinto et al. (1974) found ~~that~~ fat% in 100 samples of milk containing 2-7% fat analysed by Rose-Gottlieb and Gerber methods using 11.0 ml and 10.75 ml pipettes. The smaller sized pipette is recommended for its better concordance with Rose-Gottlieb results.

Rangi (1976) developed a method which avoids centrifugation in Gerber method. He held the butyrometer at $82^{\circ} \pm 2^{\circ}C$ for 30 min and found highly significant correlation between the two methods.

Bijeljac (1977) compared the methods for milk fat determination. Fat content was estimated in 30 samples of bulk milk by (i) the Gerber method using a 11.0 ml pipette, (ii) the modified Gerber method using a 10.75 ml pipette, and (iii) the Rose-Gottlieb method. Mean values with standard deviation (s.d.) were for (i) to (iii) respectively (%) -3.96 $\pm$ 0.704, 3.87 $\pm$ 0.704 and 3.88 $\pm$ 0.704. It was reported

that none of the differences were significant, and (ii) giving results ~~were~~ more close to those of (iii) was found to be more accurate than (i).

Detergent Tests

The most widely used tests for the determination of milk fat, the Gerber and Babcock methods involve sulphuric acid. Many attempts have been made to replace this unpleasant reagent, by alkaline or neutral reagents, surfectants etc.

Schain (1949) observed that surface active detergents lower the interfacial tension, form a complex with protein and break up the emulsion. The fat liberated can thus be measured. In his original test he used two solutions, (a) Super saturated solution of Oil Red O in isopropyl alcohol added to a mixture of standardised non-ionic detergent polyoxy-ethylene sorbitan nonolaurate in 90% ethanol, (b) standardised anionic detergent dioctyl disodium phosphate. Mixing the reagent (b) with milk and heating to 180°F effected a dispersion of the protein layer around the fat globules. The separation of fat was not complete, until the hydrophilic non-ionic detergent (a) was added, which prompted the rising of fat.

Gentillini (1950) described a volumetric method for the determination of milk fat without centrifuging. Into a Gerber buturometer, 11 ml of 60% sodium salicylate, 1 ml of isoamyl alcohol and 10 ml of milk are taken. It is shaken vigorously, then completely immersed in a water bath at 65°C

for 5 min and fat separation is effected by keeping in water bath at 80°C for 105 min.

Rao et al. (1964) evolved 'Brisk reagent' for the determination of fat in milk and milk products. The reagent consists of the following composition:

i)	Sodium hydroxide	5 g
ii)	Sodium chloride	4 g
iii)	Sodium carbonate	1 g
iv)	Sodium salicylate	4 g
v)	Sodium citrate	4 g
vi)	Borax	1 g

These are dissolved and volume is made up to 100 ml with water. To this solution, 25 ml methanol, 25 ml ethanol and 13 ml isobutenol are added. For the determination of fat, 11 ml of this reagent is added to 11 ml of milk in Gerber butyrometer and the process is proceeded by the usual method.

Sasvari and Varsanyi (1969) developed a method in which 20 ml of milk was mixed with 40 g of urea and 32 ml water, heated to 70°C to effect solution and maintained at 70°C until withdrawn into Gerber butyrometer. The tubes were placed in a water bath at 80°C for 5 min, then centrifuged for 10 min and the supernatant layer read.

The important part that the volumetric methods play in dairy economy had led to study of factors influencing the accuracy of these methods.

Roeder (1940) discussed in detail the magnitude and nature of error which can occur in the practical operation of Gerber test. The various sources of error enumerated by him are as follows:

(a) Customary tolerance in the gradation of glass,
(b) Variation in temperature of milk,
(c) Purity and quality of H_2SO_4 employed,
(d) Speed and duration of centrifugation, and
(e) Various factors such as parallax, falling temperature and movement of fat column.

Schwarz and Woerner (1960) discussed some factors influencing the results by Gerber and gravimetric determination of milk fat. They varied the composition of milk fat by addition of fatty acids, triglycerides, amyl ester of fatty acids etc., substances which might be found during a butyrometric determination of milk fat. The alteration in fat number caused by these additions did not always agree with those given by gravimetric methods. The fat content determined by Gerber method increased with the temperature and duration of shaking. The fat numbers were constant in the absence of amyl alcohol. This comparative study revealed that excess of these substances produced during the Gerber test are not strictly fat and these may be responsible for the inaccuracies in the method.

III. Instrumental methods :

There has been speedy progress in the development of instrumental methods for milk analysis in the recent years,

though efforts were made to determine milk fat on the basis of physical properties (of milk) since the early days of milk analysis. These methods are very quick and require a small quantity of milk sample.

Brio and Vladavetes (1951) described a method based on close relationship existing between degree of transparency and concentration of butterfat. A 10 ml milk sample is heated in a water bath at 40°C, cooled to 25°C, one drop of saturated sodium hydroxide added, the sample stirred and examined in a photoelectric absorptiometer graduated directly in terms of the butterfat percentage. When compared with Gerber method, it gave results which in 75% of the cases deviated within ±0.15%.

Roeder (1959) evolved a method which is based on the determination of light transmission of the samples after suitable treatment involving homogenization by ultra-sonic vibrations and the addition of ammonia to reduce the interference of colloidal particles.

Tyunilyainen et al. (1961) designed an electric apparatus for the estimation of milk fat. The apparatus was suitable for testing milk with 3.5% fat. Its operation was based on the change in electrical conductivity of milk. The absolute error was found to be 0.1 to 0.2%.

Williamson's (1962) method comprises the addition of an alkaline solution to the measured volume of milk. A radioactive compound, having a higher stability in milk fat

than in the aqueous phase is next added and the mixture is centrifuged to separate the fat phase. A portion of the aqueous phase is separated and the radioactivity measured. From this measurement, the milk fat content can be calculated.

Konev (1963) suggested the possibility of estimating milk fat from the intensity of secondary fluorescence. Secondary fluorescence emission at 550 mu is measured using an excitation wavelength of either 404 or 366 mu. In tests of samples of milk in cans, the s.d. from Gerber results was 0.08%.

In 1968, Butov proposed a rapid method which consists of the addition of 25 ml of water to 0.5 ml of milk, followed by 1 ml of a 0.04% solution of flurochrome (Fosfin 3R) and 1 ml of 1.5% sodium hydroxide and transfer to a 5 mm cuvette. Fat content is found by measurement of fluorescence at 510 nm and reference to a calibration curve prepared from milks of known fat content.

Brusilovskii and Aristova (1975) also described an instrument which measures the fluorescence produced by the fat globules of milk previously strained by addition of the dye-4-amino-N-methyl-phthalimide. Readings are made at $37 \pm 0.2^{\circ}C$. The s.d. from Rdse-Gottlieb method was $<0.1\%$ fat. Results were unaffected by the use of HCHO as preservative.

Kozlova et al. (1978) used fluorimeter for the rapid determination of milk fat. Milk is heated for 30 sec in a boiling water bath with phosphine 3RHCl and the intensity of

fluorescence of the fat phase is measured in the range of 500-560 nm, after excitation at 420-450 nm. Mean deviation from Gerber results was 0.04% for milk.

Beitz et al. (1977) developed a nephelometric method in which fat is aggregated with an emulsion by addition of an aqueous surfectant solution consisting of 5% IGEPAL DM-970 in water. Correlation of this method with Babcock method was 0.95.

Infra-red Milk Analyser

The infra-red method for analysis of milk was developed by J.D.S. Goulden of the National Institute for Research in Dairying, Reading, England. He demonstrated in 1961 that difference between the IR spectrum of water and homogenised milk at 5.73 mu, 6.46 mu, 7.9 mu and 9.6 mu could be used to estimate % of fat, protein, SNF and lactose in milk (Biggs, 1972).

In this instrument, the absorption of IR energy by carbonyl group in the ester linkage of fat molecules, by peptide linkage between amino acids in protein molecules and by alcohol groups in lactose molecules, is measured. It is specific for measurement of intact fat, protein and lactose in milk.

The most significant errors of the method were from variation in water content of different milks but these errors

could be subsequently reduced by calibrations for fat and lactose to allow for expected errors due to water, and by correcting each protein analysis after having determined the variations in water content due to change in fat percentage. Instrumental calibrations are achieved initially for adjusting results to conform to % found by standard analytical methods, with groups of milk having wide variations in component percentages.

With Mosk 2IRMA, analysis of fat, F+P, or F+P+L and SNF can be completed in 20, 27 or 33 sec, sample volume being 13-15 ml. Repeatability of the instrument for F, P and L is of 0.03%.

A mini-IRMA instrument for rapid automatic determination of F, P and L in preheated milk samples was developed (Anonymous, 1977). A complete analysis for the 3 components takes 90 sec and the results appear in digital form. The results showed the s.d. for fat ±0.035% against Mojonnier method.

White et al. (1978) developed IR reflectance analyser for measurement of fat and total solids of dairy products, to be successful in case of the liquid dairy products examined. The instrument can also measure protein and can be used for dried products.

Milko Scan 300 is capable of analysing 300 samples/hr for F and P. The s.d. between this and chemical method was ±0.06% using individual samples.

Juarez et al. (1978) used Milko-Scan 203 (A/SN Foss Electric, Denmark) to determine F, P and L. The mean and s.d. of the difference between the duplicate determinations were 0.012 ± 0.011% for fat (69 samples), 0.007 ± 0.009% for protein (68 samples), 0.008 ± 0.006% fot lactose (68 samples) and 0.014 ± 0.011% for TS (828 samples). The mean and s.d. of difference from reference methods (Gerber, Kjeldahl, Chloramine-T and gravimetric) were 0.025 ± 0.058% for fat, 0.014 ± 0.057% for protein, 0.014 ± 0.067% for lactose, and 0.048 ± 0.139% for TS. The differences from reference methods were not significant.

Sasano et al. (1980) tested the accuracy and practicability of Milko-Scan 203. For 3 milk samples each analysed 50 times, s.d. of measurements were 0.13 - 0.15 for fat %, 0.006 - 0.011 for protein % and 0.011 - 0.019 for lactose %. For 225 milk samples, correlation coefficient between estimates of F, P and L% by Milko-Scan and official Gerber, Kjeldahl and Lane-Eynon method were 0.962, 0.922 and 0.710 (all $P < 0.01$). Decreasing milk temperature from 60 to 10°C increased estimates, especially that of fat content, and optimum temperature was 40°C. In this instrument, milk fat, protein and lactose are estimated from IR spectra at 5.73, 6.46 and 9.60 um respectively and 225 samples per hr can be analysed.

Milko-Scan 104, the IR milk analyser manufactured by N.Foss Electric A/S in Denmark was intraduced in the Bremerland dairy in June 1979 to replace Milko-Tester Mark III. Heinrich (1979), Rasmussen (1980) showed that the instrument can be used to determine F, P, L, TS and SNF. Reusel (1980) used it in about 20 different experiments to analyse various milks and dairy products. Reproducibility of results was generally good (s.d. = 0.02%) being best for protein and worst for fat. Instability of fat phase often resulted in its being under-estimated. The accuracy of the results was dependent on calibration, but depended to a large extent on the intrinsic variability in composition of the samples. Accuracy of the analyses made varied from 0.02 - 0.11% for fat compared with Rose-Gottlieb, from 0.01 - 0.04% for total N compared with Kjeldahl, and from 0.04 - 0.07% for lactose compared with chloramine-T method. Lorenz and Hoffer (1980) found a correlation coefficient between TS determined by this analyser by summation of separate values and by moisture determination, respectively were 0.9947 and 0.9473. They also studied effects of preservation of milk with increasing quantities of sodium azide or calcium dichromate on results.

Voort (1980) assessed Milko-Scan 104 by using herd, individual cow, commercial and composite random milks, and produced mean differences and s.d. of difference, of 0.02% and $\pm$ 0.02% for reproducibility and 0.05% and $\pm$ 0.06% for accuracy. Results outside these values were encountered in

the individual cow milks for which the instrument was not specifically calibrated. The average molecular weight of the milk fat was an important variable. Linear regression analysis of chemical vs instrumental results showed good linearity and provided standard error (s.e.) of the estimate of the same magnitude as the s.d. of the differences. A separate assessment of the instrument for within and between day variations showed no practical significant differences. Based on overall results, the Milko-Scan 104 was judged to be capable of meeting the newly set AOAC specifications for fat, protein and lactose analysis.

The Multispec M (Berwind Instruments, Osbaldwick, York, UK) is an IR analyser for determining the fat, protein, lactose and TS content of milk and dairy products. Samples must be heated to 40°C before analysis. The instrument was tested by measuring fat and protein in 312 and lactose in 294 bulk milk samples (BMS) and comparing with standard methods, viz. Rose-Gottlieb for fat, Kjeldahl for protein and IDF method for lactose. Relative to these standard methods, the s.d. of differences in results were 0.028% fat, 0.032% protein and 0.038% lactose with correlation coefficient of 0.995, 0.998 and 0.942 respectively. Preservation of samples for 3 weeks at 4°C with $K_2Cr_2O_7$ or $NaNO_3$ had no effect on results. Interference from lactic acid (which absorbs IR energy at the fat and protein wavelengths) could be eliminated by using fresh or preserved samples. When normal milk was

analysed, there was no significant carry over effect between samples, Finland (1980). The automatic version of Multispec IR analyser is capable of carrying 1425 determinations/hr for fat, or 300 determinations/hr for fat and protein, or 225 determinations/hr for fat, protein, lactose and dry matter jointly, or of 175 determinations/hr for these constituents separately. The results are digitally presented. Samples of less than 6 ml are used and s.d. for fat, protein and lactose determination is 0.06%, Milch (1980) (cited DSA 1981, Vol.43, No. 8).

Biggs (1978) reported that in a collaborative study of AOAC (Canada), 36 milks pre-analysed by accepted standard methods were analysed with Milko-Scan instruments at 6 labs. Compared with liquid chromatography lactose determination and Mojonnier SNF determination, the Milko Scan showed an s.d. of accuracy of 0.083 and 0.073 respectively.

Clemmensen (1980) reported that variations in Milko-Scan reading at 5.73 um, caused by fat composition, are largely overcome by using a wavelength of 3.4 mu, which measures absorption of C-H bonds instead of ester bonds. In tests on 125 milk samples, he observed an s.d. of results between Rose-Gottlieb and Milko-Scan to be only 0.06% when measured at 3.4 um (Milko-Scan 203 B) vs 0.131% at 5.7 um (Milko-Scan 300) and that NaCl concentration has a greater influence on measurements at 3.4 um than at 5.7 um.

Nexo and Frandsen (Denmark, 1980) applied for patenting an instrument for analysing samples of liquid products, particularly liquid milk, for fat, protein and lactose and optionally also for TS and SNF. Its operation is based on the ability of these components to absorb IR radiation of certain frequencies. For liquid milk products, the frequency bands are within the range 4.5 - 10 um.

Milko-Tester

G. Havgard in 1959, a Danish Scientist, published a photometric method for fat determination in milk. The Foss Electric Co., Denmark began selling the Milko-tester in 1962. It measures the light scattered by fat globules in a diluted milk sample, the amount of scattering is dependent on number and size of fat flobules. If the size distribution be uniform, the amount of light reaching the photocell will be proportional to the fat content. The apparatus incorporates a universal photometer and a digit printer. Analysis are performed on 1.5 ml samples diluted 10-fold with the diluent. The s.d. for milk and homogenised milk was found to be ± 0.07% (Thamm, 1977).

Kaprazil et al. (1977) analysed 600 cow milk samples for fat content using in parallel (i) the Gerber method and (ii) Milko-tester mark III (A/S N. Foss Electric, Denmark), 400 samples were preserved with $K_2Cr_2O_7$ and 200 with Gumasin. The s.d. of (i) was ± 0.04%, of (ii) was ± 0.03% and s.d. between (i) and (ii) was ± 0.05%. The method of sample

preservation did not affect the results. They concluded that (ii) was as accurate as (i) and was simpler, safer and more rapid. However (ii) should not be used with milk samples of titrable acidity more than 11°SH, and that the shift value should be checked daily.

Wolfschoon (1979) tested Milko-tester against the Gerber method for 49 milk samples and the correlation between the two was found to be 0.998. The s.d. of the difference between milko-tester and Gerber method was 0.0595%. Reproducibility was better with milko-tester than with Gerber method. Preservation of samples for 3 days at ambient temperature (20-23°C) using $K_2Cr_2O_7$, $HgCl_2$ or HCHO did not distort subsequent results.

Mathur and Swendsen (1979) attempted to modify Milko-tester MK-III for buffalo milk, by modifying the digitalizer so as to allow for adjusting the B-value and thereby overcome the difficulty in adjusting the shift value (SV). The adjustment in B-value for cow's milk is B = 0.73 and for buffalo's milk B = 8.85 and the standard variation for both will be 1.72.

Shipe and Senyk (1980) evaluated the Milko-Tester minor against the recommended AOAC performance specifications. Results were compared with the Babcock method and other approved instruments. They concluded that the MT minor conformed to the specifications for transfer errors, linear response and homogenizer efficiency.

Wagner (1980) developed statistical techniques to calibrate ultrasonic velocimeters, formerly known as the Darison, so that standard data could be used interchangeably between different instruments. They concluded that the accuracy of fat and SNF determinations in milk was not affected by the use of cross-calibration with temperature compensation.

Shipe and Senyk (1980) evaluated performance specifications for measuring milk fat by turbidimetric means. Precision between duplicates of $<0.03\%$ for 0-5% and $<0.04\%$ for 5-8% fat has been recommended regardless of the type of milk.

Use of liquid scintillation counter in the quantification of colored solutions has been made possible by the insertion of a sealed radioactive standard of known count rate into the centre of a standard liquid scintillation vial, the colored solution is placed in the space between the outer vial and sealed standard. Using this procedure, Noble and Shand (1980) devised method for analysing milk. The protein content was determined from the formation of a blue biuret complex, fat by formation of an opalescent emulsion containing a water soluble yellow dye, and lactose by the formation of a yellow lactosazone. For fat and lactose analysis, the decreased count rate of a C^{14} scintillation source was measured and for protein analysis a ^{3}H source was used. Results thus obtained agreed well with those obtained by standard methods.

Methods for estimation of total solids and solids-not-fat :

I. Gravimetric methods

Richmond (1920) (cited Davis and Macdonald) found that by spreading small quantities of milk over a large surface during evaporation, leaves a nearly white residue, which is then dried to a constant weight.

Schulz et al. (1957) used filter paper in aluminium foil dishes, in place of the usual type of dish with sand. This procedure was found to be rapid as well as accurate and required lesser manipulation.

International Dairy Federation (1962) gave a standard FIL-IDF-21, 1962 for the estimation of milk total solids, in which 2 ml of milk is weighed in a pre-weighed and dried metal dish with a well fitted lid. It is then heated on a boiling water bath for 30 min and at 102°C for 2 hr, the dish is redried for 1 hr at 102°C until two successive weighings differ by < 0.5 mg.

Bakalor (1964) reduced the weight of the sample in the standard method from 5 g to 3 g. The accuracy of the method was not affected by this reduction and the drying time was reduced considerably in the mechanical convection oven (100 ± 1°C) used by him, after evaporation on a water bath. Absorbant discs (filter paper) and the addition of formaldehyde before evaporation respectively reduced and increased results to a significant extent.

Lunder (1971) modified the Mojonnier method in which, following the extraction of milk fat, defatted solids may be estimated by drying the residue which remains in the extraction flask after the solvents are evaporated.

II. Volumetric methods (Lactometric methods)

Though gravimetry is the standard method for precise determination of TS/SNF, for routine analysis, lactometric methods are used, which are very rapid and quick methods.

Behrend and Morgen (1879) (cited by Ystgaard et al.) were probably the first to recognize the relationship existing between the total solids (TS) and specific gravity of milk. Fleischmann (1885) appears to be the first individual to present a formula relating fat percentage, specific gravity and total solids that was sufficiently accurate to be generally accepted.

Richmond (1895) first published his equation relating total milk solids with specific gravity of milk and the fat percentage.

Since then various formulae have been developed with the object of obtaining greater accuracy. Richmond's formula has stood the test of time and is still in current use, throughout the world. It was adopted by the British Standards Institution in 1937 as a basis for their specification No. 734.

The original Richmond's formula was as follows:

$$T = 0.25\ G + 1.2\ F + 0.14 \qquad \ldots\ (i)$$

where,

T = % total solids by weight in milk.

G = 1000 (S-1) where S is the specific gravity at 15.5°/15.5°C of the milk.

B.S.I. Formula : BS:734(Part II) - 1959.

When the specifications for density hydrometry were prepared, advantage was taken of the opportunity not only to change the basis of hydrometry from specific gravity to more suitable density but also to adopt the technique of preheating the milk to eliminate Recknagel contraction.

The specific gravity 15.5°C/15.5°C of a milk may be expressed in terms of its density in g/ml at 20°C (say d), with the help of coefficient of cubical expansion of milk and the density of water at 15.5°C and

$$S = d + 0.00205 + 0.00005\ F \qquad \ldots\ (ii)$$

where the fat in the milk is in the liquid stage, substituting in (i) the value of S given by (ii), we have

$$T = 0.25 \times 1000\ (d + 0.00205 + 0.00005\ F - 1) + 1.2\ F + 0.14$$

$$= 0.25\ D + 1.2125\ F + 0.6525$$

where D = 1000 (d-1)

a simplified version of this equation formed the basis of the

calculation of percentage SNF in BS:734 1955, namely

$$SNF = T-F = 0.25\ D + 0.21\ F + 0.66$$

the fat being in the liquid state. This equation was revised to $SNF = 0.25\ D + 0.22\ F + 0.72$ and when the fat is in solid state

$$SNF = 0.25\ D + 1.22\ F + 0.55.$$

Richmond (1894) suggested a separate formula for buffalo milk $T = 0.27\ \frac{G}{D} + 1.191\ F$, while Babcock (1891) gave the following well-known equation:

$$T = 1.2\ F + 0.25\ L$$

Sharp and Hart (1936) recommended measuring the specific gravity after heating the milk to 45°C for 90 sec and cooling to 30°C to bring the fat in entirely liquid state. ISI recommended the heating of samples to 40°C for 5 min and then bringing them to the temperature of determination.

Giribaldo and Peluffo (1941) gave an indirect method of calculation of dry matter in cow's milk and they suggested the following equation:

$$TS = 0.282\ (density - 1) + 1.19\ F$$

Reuda (1943) recognised that a correction must be applied to the ordinary lactometer reading before they could be used in the Sharp and Hart equation. He determined the magnitude of the correction by comparing the actual reading of a lactometer in a sucrose solution at 30°C, with the actual

reading of a lactometer in a sucrose solution at 30°C, with the actual specific gravity at 30°C/30°C as determined by a pycnometer. He obtained a correction of 3.2°Q which differs 0.2°Q from the value calculated.

Desai and Patel (1945) found from an analysis of 16 samples, a discrepancy ranging between 1.3 to 3.8 % of total solids between gravimetric results and those obtained by the Richmond's formula, and the discrepancy was increased by addition of water or abstraction of fat.

Ramachandran et al. (1953) studied the applicability of Richmond's formula by analysing 1529 individual and herd samples for cow and buffalo milk and they reported that the calculated values were both higher and lower than the gravimetric method by upto ± 1.0. Only in 40% of the samples, the agreement was within ± 0.25. When the formula suggested by Richmond for buffalo milk was used, they obtained less satisfactory results than the commonly used formula.

Heinemann et al. (1954) calculated total solids on 78 samples by means of Babcock formula and Sharp and Hart formula modified by Herrington (TS = 0.2594 x (Q at 30°C + 3.0)+ 1.2648 x F) and compared with the Mojonnier total solids. The regression equation was shown to substantiate the partial regression coefficient in the Sharp and Hart equation modified by Herrington (1946). They also found that the protein content of the milk influenced the accuracy of the total solids determination by lactometer.

Watson (1957) designed a new type of lactometer which was calibrated to read the specific gravity of milk at 102°F. The approximate dimensions in cm were as follows: total length 15, stem length 6.5, stem diameter 0.5, bulb diameter 3.5 and scale length 0.23. The scale is designed for whole milk over the range of 25.5 to 33.5 degrees, the smallest division being 0.5°. Using this lactometer, he presented a method for the calculation of per-cent solids in milk from the percentage of fat and the lactometer reading at the temperature of 102°F.

$$T = 1.254\ F + 0.271\ L - 0.216$$

Gupta and Dastur (1959) studied the estimation of SNF in milk by the modified Richmond's formula and reported that the s.d. of results from the gravimetric values was ± 0.10% SNF in the sample of cow's milk and ± 0.11% SNF in bufallo's milk, taking density reading at 20°C. Using 27°C as the temperature at which the density was measured, the s.d. were ± 0.11% and ± 0.12% SNF in cows and buffaloes milk respectively.

Rowland and Wagstaff (1959) assessed the accuracy of the method specified in B.S. 734 : 1937 for the estimation of total solids and SNF of milk, from the density and fat contents, with the gravimetric method by analysing 2425 samples of milk. They observed that the % of solids estimated by the density method was lower, by an average of 0.06, than those determined

gravimetrically. To correct this error, they proposed the following density formula:

$$TS = 0.25\ D + 1.22\ F + 0.72$$

$$SNF = 0.25\ D + 0.22\ F + 0.72.$$

These were adopted in October 1957 in an amendment to BS : 734 (1955).

Edward (1961) compared the hydrometric and gravimetric methods for the determination of SNF, when applied to the milk of individual cows, by analysing 304 samples and recorded high differences between the two methods, the magnitude of difference varied with the season and the stage of lactation. The main factor influencing the magnitude of difference was the protein % of milk, fat having a small but significant effect, while lactose and ash content had no effect. It was concluded that hydrometric methods were not suitable for determining SNF of individual samples accurately, although the accuracy was improved by the use of a correction factor for protein %.

Erb and Manu (1962) and Wallace (1963) pointed that a single regression equation of indirect SNF estimation is subject to errors other than those inherent in the methods of analysis, these errors being primarily due to variation in the lactose/protein ratio, may be appreciable (s.d. of 0.2% SNF) with the milk samples from individual cows and will vary with the stage of lactation.

Vyas et al. (1973) examined the accuracy of Watson's formula to estimate the total solids in milk of Kankrej cows and Surti buffaloes and found insignificant difference in total solids by Watson's formula and the AOAC method. They observed that the Sharp and Hart's formula and Richmond's formula were less accurate than the AOAC method.

Pruthi and Bhalerao (1973) examined the suitability of Richmond's formula as in BS : 734 (1959) for the SNF estimation in buffalo milk against the gravimetric method and observed that the Richmond's formula gave lower SNF values than the gravimetric method and difference was found to increase with an increase in fat as well as SNF percentages.

Khalifa (1974) examined the applicability of Richmond's formula and found lower values by 0.07 as compared to gravimetric method. His modified formula is:

$$TS = 0.25\ L + 1.21\ F + 0.21.$$

Sebastian et al. (1974) modified Richmond's formula using Zeal lactometer at 29°C and proposed the following equation:

$$SNF = 0.25\ L + 0.2\ F + 0.50.$$

Sen (1977) modified the formula for SNF calculation by correcting the lactometer reading, by addition of 0.2 lactometer degrees to the reading for each degree rise in temperature above 60°F, when the temperature ranged from

80 to 92°F. The formula was:

$$SNF = 0.25\ CLR + 0.2\ Fat - 0.16,$$

where, CLR = corrected lactometer reading.

Mehta and Vyas (1977) made a comparative study of various lactometric methods to estimate total solids in milk of Jersey cows by analysing 100 milk samples using 4 lactometers: (i) Watson's, calibrated at 102°F, (ii) the Avon lactometer manufactured in Germany and calibrated at 84°F, (iii) the Zeal lactometer, manufactured in the UK and calibrated at 84°F, and (iv) the Desko lactometer, manufactured in India and calibrated at 60°F. The per-cent of total solids was calculated using Sharp and Hart's formula with (ii) and (iii), and using the Richmond's formula with (iv). Results using (i) were highly correlated (r = 0.99) with those obtained using the time consuming standard AOAC method for both morning and evening samples. Methods (ii) and (iv) did not give satisfactory results.

Patil and Kulkarni (1978) modified the Richmond's scale to give a direct value of SNF in milk. The scale is based on the ISI formula for the calculation of SNF from CLR, which eliminates one substraction operation while calculating the payment for milk based on SNF content.

Patel and Gandhi (1980) advocated the standardisation of the formulae for calculating SNF from lactometer reading (L) and fat % (F) and they found close agreement between the results

of gravimetric method and those by using at 60°F of a lactometer calibrated at 60°F with the help of the formula

$$SNF = 0.25\ L + 0.2\ F + 0.14$$

Rao and Bector (1980) conducted a comparative study on the SNF determination in milk by calculations. They tested the applicability of (i) Richmond's formula (SNF = 0.25 CLR + 0.2 F + 0.14) using Quevenne sp. gr. lactometer, (ii) modified Richmond's formula (SNF = 0.25 L + 0.2 F + 0.50) using Zeal sp. gr. lactometer, (iii)BSI formula (SNF = 0.25 CLR + 0.22 F + 0.72) using density hydrometer along with fat, estimated by Gerber method using a 10.75 ml pipette for calculation of SNF, by analysing 86 samples each of cow's and buffalo's milk. In all the milk samples analysed, the calculated values of SNF by the 3 methods were either higher or lower than the gravimetric values, indicating that these methods do not give a true estimate of SNF in milk.

Bector and Sharma (1980) modified the Richmond's formula so that SNF values are in close agreement with those from the gravimetric methods and gave a correction table which when used with either the modified Richmond's formula or with the Richmond's formula gave reliable results.

Indian Standards Institution in its standard IS:1585, 1980, gave the specifications for a new sp. gr. lactometer calibrated at 27°C and suggested the following

formula:

$$SNF = 0.25\ L + 0.25\ F + 0.60$$

where, L = new lactometer reading at 27°C, and

F = fat percentage.

III. Instrumental Methods

Several instrumental methods have been developed from time to time, by different workers.

Vizhintaite (1964) developed an IR method for the total solids estimation in milk. The results showed the deviation from the standard drying method of ± 0.13%.

Ciusa and Barbiroli (1970) applied the Pulsor apparatus for rotation vacuum drying of liquids manufactured by Ascenso (Milan) to determine TS in 1 ml samples of whole milk. Reproducibility is said to be ± 0.2% TS.

The 3 milk components viz., fat, protein and lactose have the absorption at 5.73 um arising from carbonyl group of the triglyceride structure, 6.46 um due to the amide group in the peptide linkage of intact protein, 9.60 um due to alcohol groups respectively, and the absorption due to SNF takes place at 7.9 um. These facts form the principle of IR analysis of milk by Infra-red Milk Analyser (IRMA), which allows the direct determination of fat, protein, lactose and SNF (Hall, 1975). The fundamental component of an IRMA is a double beam infrared spectrometer which compares the absorption of a milk sample at the above-mentioned wavelengths with the absorption of pure water.

Juarez et al. (1979) used the mini-IRMA to determine TS in liquid milk by measurement of a band in the spectrum of water at 4.8 um. The precision was assessed from an s.d. calculated by the method of Kalt hoff and Sandell (Quantitative Chemical Analysis (1969) Mac Millan). They concluded that except for lactose and TS in dried skim milk, results with the mini-IRMA are considered acceptable.

Oshima and Takahashi (1979) developed a new method for TS estimation based on the electrical conductivity of milk. Their method, however, is applicable to normal milk from individual healthy cows only.

Takahashi et al. (1978) made measurements of total milk solids by microwave heating. In their method, 1 g of milk sample is applied to a glass fibre filter (26 mm diam.) and dried quickly in the drying unit which consists of a microwave generator, wave-guide type oven, electrobalance and indicator unit. The sample is positioned on the scale stand of the electrobalance within the drying unit and weight before and after drying is displayed digitally. Fifty tests on samples from the same batch of milk showed an s.d. of 0.018% TS. Maximum deviation from results by the official method was 0.05% TS and average value for TS was 12.164% vs 12.150% by the official method. Testing time was reduced from 8 hr to 3.5 min.

The CEM Corporation's AVC moisture/solids Analyser consisting of a microwave drying system, an analytical

electronic balance and a microprocesser-based digital Computer (Collins, 1979).

Bossuyt (1980) tested the suitability of Milko-Scan 104 and DMA 46 and reported that these gave good agreement on average with reference methods for fat, protein, lactose and TS determination, and that recalibration of the instrument (e.g. weekly) seemed essential with a mixed sample representative of the samples for analysis.

Green and Park (1980) compared microwave, AOAC and vacuum oven methods for TS determination. The regression equation between the AOAC (Y) and microwave (X) results was found to be $Y = 1.00778\ X - 0.29495$. They attributed the consistently higher results by the microwave methods to browning in the AOAC method.

Thomasow and Pasche (1980) used the microwave 'Apollo, Mark III' Instrument (Photovolt Corporation, New York) to determine the TS in milk products. They concluded that the instrument gives in general results in good agreement with those by standard methods.

Hayward and Knopf (1980) investigated the sample position effects on moisture analysis by a microwave oven method and reported that position in the oven significantly affected moisture content measurements. They recommended that the samples should be placed in the same position in the oven for each determination, so as to obtain uniformity in results.

Asker et al. (1977) evaluated some rapid techniques for determining milk total solids, and reported that the rapid techniques can be used with a reasonable degree of repeatability.

Boon (1979) compared various methods for determining TS of milks and skim milk. Concentrated skim milk and reconstituted dried whole or skim milks were analysed for TS content using the following methods: (i) the British Standards Method, (ii) the Mojonnier vacuum oven method, and (iii) the method of McDowell. Method (i) had greatest reproducibility, method (ii) gave the lowest overall mean result, although not significantly different from those obtained with (i) and method (ii) gave a significantly higher value than (i) or (ii).

CHAPTER IV

MATERIALS AND METHODS

MATERIALS AND METHODS

Milk Samples

Milk samples from individual animals (cows and buffaloes) were collected from the herd maintained at the Institute. Fresh skim milk and cream were collected from the Experimental Dairy of the Institute to prepare milk samples of desired fat percentages by mixing in different proportions of skim milk and cream. Fat percentage of milk samples thus obtained had a range from 1.35 to 11.5.

Treatment to the Samples

All milk samples were heated to 40°C and held at that temperature for 5 min to ensure complete liquification of milk fat globules and thoroughly mixed avoiding incorporation of air. The samples were then brought to 27°C (the temperature of determination) and then lactometer readings were taken.

Analysis of Milk Samples

All milk samples were analysed, in duplicate, for the following parameters:

1. Fat by Gerber method,
2. Total solids by Gravimetric method,
3. Solids-not-fat by subtracting the Gerber fat (fat percentage as obtained in Gerber method) from the total solids estimated by the gravimetric method.
4. New lactometer reading at 27°C.

1. Estimation of fat by the Gerber method

The fat content was determined according to the method described in IS:1223 (Part I, 1979, Part II, 1972).

From the prepared and well mixed milk samples, 10.75 ml was pipetted into the calibrated butyrometer containing 10 ml of sulphuric acid of desired strength (1.81 g/ml) without mixing the contents. Then 1 ml of isoamyl alcohol was added from the automatic pipette. The butyrometer was stoppered and the contents were mixed thoroughly by inverting the butyrometer 2-3 times and then it was kept in the waterbath maintained at 65°C for at least 5 min keeping the scale upward and completely immersed in water. Then the butyrometer was kept in the centrifuge and centrifuged at 1100 r.p.m. for 4 min. The temperature of the butyrometer was again adjusted to 65°C by placing it in waterbath for at least 3 min. Then fat column was adjusted within the scale on butyrometer and readings were taken from the graduated scale using a magnifying lens.

2. Determination of total solids by Gravimetric method

This was estimated by the oven-drying method as described in IS:1479 (Part II, 1961). The method, in brief, is as follows:

The clean dry empty dish (made of aluminium alloy, 7 to 8 cm diameter and about 1.5 cm in height) with lid was weighed accurately. Five ml of prepared sample was pipetted

into the dish and weighed accurately, with the lid on. The dish was kept horizontal to promote uniform drying and protected from direct contact of the water bath. After at least 30 min, the dish was removed, the bottom wiped and transferred to the oven maintained at 98-100°C placing the lid by the dish. The dish was transferred after 3 hr. It was allowed to cool for about 30 min and weighed. The dish was returned to the oven and uncovered, and heated for 1 hr. It was then removed to the desiccator, cooled and weighed as before. The process was repeated till the loss in weight between successive weighings did not exceed 0.5 mg. The lowest weight was noted.

$$\text{Total solids per cent by weight} = \frac{100\ w}{W}$$

where, w = weight in gm of the residue after drying
W = weight in gm of the prepared sample taken for the test.

3. Solids-not-fat were determined by subtracting the Gerber fat from the total solids estimated by the gravimetric method.

4. <u>Determination of Lactometer reading at 27°C using New Specific Gravity Lactometer</u>

The well mixed sample of milk was gently poured down the side of a metal cylinder (3.5 x 20.0 cm) which was kept in a water bath and the temperature of the sample was brought

to 27°C. The cylinder was filled with milk so that any foam present could be blown off. The lactometer and cylinder were thoroughly tempered to the temperature of test. The lactometer was submerged in the milk, so that very little milk was on the exposed stem of the lactometer at the time of reading. The lactometer was read to the top of the meniscus as soon as it came to rest.

The New Lactometer

The new lactometer used in this study was designed and made at Southern Regional Station of NDRI (Rama Murthy, 1981). The following are the specifications of the new lactometer: IR:1985-1980.

Total length	=	18.5 mm approx.
Scale	=	1.020 to 1.035 sp. gr.
Sub Div.	=	0.0005 sp. gr.
Total sub-div	=	30
Accuracy	=	± 0.0005 sp. gr.
Temperature of calibration	=	27°C
Number of sub-divisions beyond nominal scale of the top graduation	=	One or nil
Scale length	=	41 ± 4 mm
Length of stem above top graduation mark	=	20 ± 5 mm
Distance below the lowest graduation mark	=	10 mm
External dia. of stem containing scale	=	4.0 mm

External dia. of bulb = 22 ± 1 mm

Maximum length of uniform stem = 80 mm

Volume in ml below bottom graduation line

(i) Not more than 37 mm
(ii) Not less than 31 mm

Calibration of Butyrometer

The calibration of milk butyrometer was checked by the method as described by Thakur Das (1965). The method, in brief, was as follows:

The butyrometer was properly cleaned with chromic acid, dried and tied with a wire loop at the neck to facilitate weighing. Then it was filled with mercury AR grade from BDH exactly up to some suitable division.

The butyrometer was weighed and was again filled to the graduation approximately one scale division below with the help of microburette and again the weight was taken. This was repeated all over the scale length. From the weight and density of mercury at room temperature, internal volume of each 1% graduation was calculated. Each 1% graduation had a volume of 0.125 ± 0.002 ml. Any butyrometer having a greater deviation was not used.

Determination of capacity of milk pipette

This was checked by the method as described in IS:1223 (Part II, 1970). The method, in brief, was as follows:

Thoroughly cleaned the pipette, dried and clamped in a vertical position with the jet downwards. Then filled with distilled water to a short distance above the graduated mark. Wiped the outside of the delivery tube jet free from water with a piece of clean filter paper and allowed the water to run out slowly, till the water level came to the graduation mark. Then placed a tared 50 ml capacity beaker below the pipette and allowed the delivery of the water into the beaker. Removed the beaker at the end of draining time of the pipette. Determined the weight of the water thus delivered. Observed the room temperature and calculated the volume of the pipette from the weight of the water and its density at room temperature from the tables. Only those pipettes having volume of 10.75 ± 0.03 ml were used in this study.

<u>To check the accucary of the new specific gravity lactometer</u>

This was done by the method described in IS :~~1183~~ 9585-1980. The method, in brief, was as follows:

Prepared liquids with a surface tension of approx. 50 dynes/cm by dissolving appropriate weight of Na_2CO_3 (anh.) in 300 ml of distilled water. Added to the solution thus obtained, 50 ml of industrial methylated spirit (92% ethanol) and made up the volume to 500 ml with distilled water.

Specific gravity (27°C)	Mass of anh. Na_2CO_3 (g)
1.019	15.3
1.026	19.2
1.030	21.6
1.034	24.0

The results obtained were compared with the above standards. The lactometer having any deviation from the standards was not used.

CHAPTER V

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

A total number of 156 milk samples (having fat and solids-not-fat (SNF) percentages varying from 1.35 to 11.5 and 7.11 to 11.74, respectively) consisting of standardised milk samples, those obtained from individual animals (cows and buffalo), as well as those prepared by mixing fresh cream with fresh skim milk, were analysed in duplicate for the estimation of SNF by the following 4 methods:

(a) Subtracting the fat obtained by Gerber method from the total solids estimated by oven drying method (SNF_G)

(b) By calculations using the formula as suggested in IS:9385-1980, using new specific gravity lactometer calibrated at 27°C (SNF_N)

(c) By calculations using the formula as developed in the present study for all the 4 types of milk samples (SNF_P)

(d) By calculations using various particular formulae developed for different types of milk (SNF_P).

The individual values of fat and SNF of milk samples as determined by the above 4 methods are presented in Appendix-I.

Checking the suitability of the Indian Standards Institution (ISI) suggested formula

Table 1 shows the range of SNF values of milk samples analysed in this study by the gravimetric method and by calculation following the ISI suggested formula. The range of differences between the SNF values as determined by the

TABLE 1

Solids-not-fat of milk samples of varying fat percentage as determined by gravimetric method and by calculation by using new specific gravity lactometer and by following the ISI suggested formula

Fat % range	No. of samples	Range of SNF(%) as determined by SNF_G (method(a))	Range of SNF(%) as determined by SNF_N (method(b))	Difference in SNF from gravimetric method (Range)
<2	6	8.15 to 8.49	7.529 to 9.500	-.285 to +1.120
2 to 3	9	8.14 to 9.56	8.650 to 9.887	-.215 to +.990
3 to 4	24	8.04 to 9.18	8.400 to 9.250	-.263 to +1.195
4 to 5	31	7.11 to 10.58	8.225 to 10.450	-1.235 to +1.560
5 to 6	22	8.32 to 10.35	8.550 to 10.150	-.4775 to +.901
6 to 7	16	8.19 to 11.12	9.050 to 10.155	-.390 to +.935
7 to 8	17	9.11 to 10.62	9.175 to 10.663	-.995 to +.780
8 to 9	11	8.47 to 10.62	8.963 to 11.050	-.566 to +1.843
9 to 10	14	7.62 to 11.67	9.500 to 11.425	-.745 to +2.205
10 to 11	4	8.31 to 8.96	8.950 to 9.675	+.040 to +.775
>11	2	8.85 to 11.74	9.025 to 11.537	-.217 to +.175

gravimetric method (SNF_G) and those determined by the volumetric method (SNF_N) are also presented.

The frequency distribution of differences in SNF of milk by gravimetric method and by calculation using the new specific gravity lactometer are given in Table 2.

TABLE 2

Frequency distribution of differences in SNF values as calculated by the new formula suggested by ISI from the SNF_G

Difference in SNF range (SNF_N - SNF_G)	Frequency
$<-.50$	8
$-.50$ to $<-.40$	2
$-.40$ to $<-.30$	5
$-.30$ to $<-.20$	6
$-.20$ to $<-.10$	8
$-.10$ to <0	6
0 to $<+.10$	15
$+.10$ to $<+.20$	16
$+.20$ to $<+.30$	21
$+.30$ to $<+.40$	18
$+.40$ to $<+.50$	11
$\geq+.50$	40
Total	156

From Table 2, it can be seen that out of 156 samples analysed, 121 showed the +ve difference with an average of +0.4351 (s.d. = 0.3570) and the rest of 35 samples showed the -ve difference with an average of -0.3303 (s.d. = 0.2770).

From the variance analysis of the data, the results obtained by calculations using ISI suggested formula (Method (b)) were found to be significantly different from those obtained by gravimetric method, in case of cow, prepared and standardised milks and in case of buffalo milk, the differences were not significant.

Analysis of data for Multiple Regression

Since the formula as suggested by the ISI for estimation of SNF in milk using new lactometer did not give its true estimate, it was thought to develop a regression equation by analysing the data for multiple regression of SNF % on Gerber fat % (F) and new specific gravity lactometer reading (L) (applied collectively to 156 samples), which gave the following equation:

$$\text{Total solids (TS)} = 1.29757497\ F + 0.282249157\ L - 0.80506707$$

and

$$\text{SNF} = 0.29757497\ F + 0.282249157\ L - 0.80506706$$

($R = 0.986$, $R^2 = 0.972$)

$$= 0.28\ L + 0.3\ F + \begin{matrix} 0.002249157\ L \\ -0.002425130F \\ -0.805006986 \end{matrix}$$

Mean values for L and F were 29.342987 and 5.75576923 respectively. These values when substituted in the above equation, gave:

$$\text{SNF} = 0.28\ L + 0.3\ F - 0.75 \quad \text{(P)}$$

and

$$\text{TS} = 0.28\ L + 1.3\ F - 0.75 \quad \text{(P)}$$

The multiple regression analysis when applied to the data for buffalo and cow milk samples, gave the following equations:

(i) for buffalo milk samples

$$SNF = 0.25306910\ L + 0.30100415\ F + 0.129539902$$

$$= (0.253\ L + 0.301\ F + 0.13)$$

$$(R = 0.96650255,\ R^2 = 0.934127761)$$

The above equation may be simplified to

$$SNF = 0.25\ L + 0.3\ F + 0.23$$

$$\text{and } TS = 1.25\ L + 1.3\ F + 0.23$$

(ii) for cow milk samples

$$SNF = 1.23895328\ F + 0.299463837\ L - 1.0306$$

$$(R = 0.921772399,\ R^2 = 0.849664356)$$

which may be simplified to the equation:

$$SNF = 0.3\ CLR + 0.25\ F + 0.98$$

Table 3 shows the SNF values of milk samples by the method (c) i.e. SNF_P and method (a) i.e., SNF_G and the range of the differences of SNF_P from SNF_G.

Table 4 shows the frequency distribution of differences in SNF of milk by gravimetric method and by calculations using the formula developed in the present study.

Out of 156 samples analysed, 79 showed the -ve difference with an average of -0.3262 (s.d. = 0.3138), and the rest of 77 samples showed the +ve difference with an average of +0.3297 (s.d. = 0.3662).

TABLE 3

Solids-not-fat of milk samples of varying fat percentage as determined by gravimetric method and by calculation by using the formula developed in this study

Fat % range	No. of samples	Range of SNF (%) as determined by SNF_G (method(a))	Range of SNF (%) as determined by SNF_P (method(c))	Difference in SNF from gravimetric method (Range)
<2	6	8.15 to 8.49	8.300 to 9.254	-.139 to +.874
2 to 3	9	8.14 to 9.56	8.308 to 9.715	-.469 to +.761
3 to 4	24	8.04 to 9.18	8.049 to 9.273	-.579 to +.928
4 to 5	31	7.11 to 10.58	7.832 to 10.379	-1.476 to +.620
5 to 6	22	8.32 to 10.35	8.242 to 10.057	-.685 to +.703
6 to 7	16	8.19 to 11.12	8.829 to 10.521	-.471 to +1.157
7 to 8	17	9.11 to 10.62	8.606 to 10.324	-1.131 to +.742
8 to 9	11	8.47 to 10.62	8.758 to 10.662	-.566 to +1.802
9 to 10	14	7.62 to 11.67	9.490 to 10.989	-1.245 to +2.122
10 to 11	4	8.31 to 8.96	8.770 to 9.586	-.131 to +.656
≥11	2	8.85 to 11.74	8.866 to 11.000	-.740 to +.016

TABLE 4

Frequency distribution of differences in SNF values as calculated by the formula developed in the present study (SNF_P) from the SNF_G

Difference in SNF range (SNF_P - SNF_G)	Frequency
$<-.50$	18
$-.50$ to $<-.40$	6
$-.40$ to $<-.30$	8
$-.30$ to $<-.20$	13
$-.20$ to $<-.10$	9
$-.10$ to <0	23
0 to $<+.10$	24
$+.10$ to $<+.20$	12
$+.20$ to $<+.30$	12
$+.30$ to $<+.40$	6
$+.40$ to $<+.50$	7
$\geq+.50$	18
Total	156

Mean values of SNF of milk samples determined by different methods

Table 5 shows the mean values of SNF of cow, buffalo, prepared and standardised samples as determined by the 4 methods.

TABLE 5

Mean values of SNF of milk samples determined by different methods

Type of milk sample	No. of samples	SNF_G	SNF_P	SNF_N	SNF_p	Critical difference
Buffalo	52	9.976	9.893	10.164	9.991	$F_{cal} < F_{tab}$
Cow	57	8.639	8.630	8.938	8.635	o.1883
Prepared	38	9.038	9.165	9.319	8.603	o.275
Standardised	9	8.633	8.384	-	9.1	o.236

From this table, it can be seen that in the case of buffalo milk, F_{cal} is not significant and hence the 4 methods of SNF estimation do not differ from each other significantly. In the case of cow milk, the difference between mean SNF_G and SNF_N, is greater than the critical difference while the differences between SNF_p and SNF_G, SNF_N and SNF_p and SNF_P and SNF_p are less than the critical difference. Thus, for cow milk, only the gravimetric results and those by ISI suggested formula are significantly different from each other.

For prepared milk samples, only SNF_N and SNF_G, SNF_p and SNF_G and SNF_p and SNF_p, differ significantly from each other while in case of standardised milk samples, only SNF_N and SNF_G, SNF_N and SNF_p, differ significantly.

Analysis of variance

The one way variance analysis of the data for buffalo, cow, prepared and standardised milk samples, showed that the results by the ISI suggested formula are significantly different from those obtained gravimetrically by subtraction of fat % from gravimetric total solids, except in case of buffalo milk. Tables 6-9 show the results of variance analysis for the 4 types of milks.

TABLE 6

Analysis of variance table for buffalo milk

Source of variation	Degrees of freedom	Total sum of squares	Mean sum of squares	F_{cal}
between methods	3	2.020	0.674	1.694*
Error	204	81.089	0.397	

* Insignificant at 5% level

TABLE 7

Analysis of varaince table for cow milk

Source of variation	Degrees of freedom	Total sum of squares	Mean sum of squares	F_{cal}
between methods	3	3.937	1.312	4.986**
Error	224	58.961	0.263	

** Significant at 1% level

TABLE 8

Analysis of variance table for prepared milk

Source of variation	Degree of freedom	Total sum of squares	Mean sum of squares	F_{cal}
between methods	3	10.794	3.598	9.617*
error	148	55.369	0.374	

*Significant at 1% level

TABLE 9

Analysis of variance table for standardised milk

Source of variation	Degree of freedom	Total sum of squares	Mean sum of squares	F_{cal}
between methods	2	0.987	0.494	7.556*
error	24	1.568	0.653	

* Significant at 1% level

The results obtained by method (c) are much nearer to gravimetric results than those obtained by the various partifular formulae for each type of milks (method (d)).

Conclusion

From the present investigation, it is clear that the formula developed in this study could be used for SNF calculation for all the 4 types of milks since the results obtained by using this formula, do not differ significantly from the gravimetric results. The ISI suggested formula for the new specific gravity lactometer could be used for SNF calculation in case of buffalo milk only.

CHAPTER VI

SUMMARY

SUMMARY

The formula suggested by Indian Standards Institution (ISI) for solids-not-fat (SNF) calculation in milk, using new specific gravity lactometer has been examined, when the Gerber fat determined with 10.75 ml milk pipette is used in its (SNF) calculation (SNF_N). The results obtained were compared with the SNF obtained by subtracting the Gerber fat from total solids estimated by gravimetric method (SNF_G). The SNF values as calculated by using ISI suggested formula were generally higher than the SNF_G and were significantly different from it, in case of cow, prepared and standardised milk samples, while in case of buffalo milk, the difference between SNF_N and SNF_G was not significant.

Since the formula suggested by ISI did not give true estimate of SNF, the data were statistically analysed and a regression equation of SNF on fat and new lactometer reading at 27°C obtained. The SNF_G values were not significantly different from those obtained by using the regression equation obtained (eq. P, page 51) the latter values being more nearer to SNF_G than those calculated using the ISI suggested formula.

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APPENDIX

APPENDIX I

Individual values of fat, new lactometer readings at 27°C (L) and solids-not-fat as determined by the different methods

Fat %	New lactometer reading at 27°C (L)	Total solids gravimetric	SNF %			
			SNF_G	SNF_N	SNF_p	SNF_P
1	2	3	4	5	6	7
		For buffalo milk samples				
4.3	32.5	14.88	10.58	9.800	9.643	9.637
4.9	34.5	15.46	10.55	10.450	10.329	10.379
5.0	28.0	13.57	8.57	8.850	8.714	8.576
5.1	29.75	14.12	9.02	9.313	9.191	9.099
5.3	26.5	13.62	8.32	8.550	8.998	8.242
5.5	28.5	14.48	8.98	9.100	8.996	8.866
5.9	30.5	15.36	9.46	9.700	9.622	9.548
5.95	30.5	15.85	9.90	9.713	9.637	9.563
5.95	32.25	16.30	10.35	10.150	10.079	10.057
6.0	30.0	15.99	9.99	9.600	9.526	9.437
6.1	31.75	17.22	11.22	10.063	9.998	9.960
6.1	28.0	14.29	8.19	9.125	9.049	8.903
6.2	33.0	16.77	10.57	10.400	10.345	10.342
6.3	31.0	15.83	9.53	9.925	9.869	9.808
6.3	33.0	16.49	10.19	10.425	10.375	10.372
6.4	30.5	16.18	9.78	9.825	9.772	9.697
6.5	27.5	15.64	9.14	9.100	9.044	8.880

contd....

1	2	3	4	5	6	7
6.7	31.0	16.55	9.85	10.025	9.989	9.927
6.8	31.5	17.26	9.46	10.175	10.146	10.098
6.8	29.0	16.13	9.33	9.550	9.513	9.393
6.8	33.0	17.31	10.51	10.550	10.525	10.521
6.9	30.5	16.73	9.83	9.950	9.923	9.845
7.0	26.5	16.50	9.50	9.175	8.941	8.606
7.0	29.0	16.515	9.515	9.600	9.574	9.452
7.1	30.5	17.60	10.50	10.200	9.983	9.905
7.1	32.0	17.36	10.26	10.575	10.363	10.328
7.1	31.0	17.62	10.52	10.325	10.109	10.0456
7.2	32.0	17.14	9.94	10.600	10.393	10.357
7.2	29.0	16.27	9.07	9.850	9.634	9.511
7.3	29.0	17.71	10.41	9.875	9.664	9.541
7.4	30.0	16.51	9.11	10.150	9.947	9.853
7.8	31.25	18.07	10.27	10.663	10.384	10.324
7.8	31.0	17.95	10.15	10.600	10.320	10.254
7.8	31.0	18.09	10.29	10.600	10.320	10.254
7.8	30.5	17.70	9.90	10.475	10.194	10.113
7.8	30.5	18.16	10.36	10.475	10.194	10.113
7.9	30.0	17.90	10.00	10.375	10.097	10.001
8.1	30.75	16.57	8.47	9.362	9.347	9.272
8.2	29.5	18.67	10.47	10.325	10.061	9.949
8.6	30.0	18.66	10.06	10.65	10.308	10.209
8.7	31.5	19.30	10.60	11.050	10.718	10.662
8.8	30.0	18.96	9.86	10.700	10.368	10.269

Contd....

1	2	3	4	5	6	7
8.8	30.5	19.42	10.62	10.825	10.495	10.409
8.9	29.0	19.47	10.57	10.016	10.145	10.016
9.0	29.0	19.52	10.52	10.500	10.176	10.046
9.1	30.5	19.65	10.55	10.900	10.585	10.498
9.3	32.0	19.98	10.68	11.425	11.025	10.981
9.5	29.25	14.54	10.04	10.913	10.389	10.355
9.7	27.5	19.46	9.76	10.300	10.007	9.831
9.8	29.5	21.47	11.67	10.925	10.543	10.525
9.9	27.0	20.14	10.24	10.10	9.940	9.749
11.5	29.75	23.24	11.74	11.538	11.118	11.000
		For cow milk samples				
2.8	29.9	11.02	8.22	8.550	8.307	8.205
2.9	26.5	11.40	8.50	8.475	7.584	7.529
3.2	28.5	11.32	8.12	8.525	8.253	8.182
3.4	28.0	11.50	8.10	8.450	8.151	8.101
3.4	29.0	12.36	8.96	8.700	8.450	8.382
3.6	31.0	12.72	9.12	9.250	9.096	9.006
3.7	27.5	11.95	8.25	8.400	8.073	8.049
3.7	28.5	12.25	8.55	8.650	8.372	8.331
3.8	28.5	12.30	8.50	8.675	8.396	8.361
3.8	30.25	12.75	8.95	9.113	8.919	8.854
3.9	28.5	12.50	8.60	8.700	8.419	8.390
3.9	29.0	12.50	8.44	8.825	8.569	8.531

Contd....

1	2	3	4	5	6	7
3.9	27.5	12.06	8.16	8.450	8.121	8.108
4.0	26.5	12.54	8.54	8.225	7.846	7.832
4.0	27.0	11.30	7.30	8.350	7.995	7.856
4.0	28.5	12.61	8.61	8.725	8.444	8.420
4.0	29.0	12.57	8.57	8.850	8.593	8.561
4.0	29.0	12.60	8.60	8.850	8.593	8.561
4.0	29.0	12.25	8.25	8.850	8.593	8.561
4.0	29.0	12.40	8.40	8.85	8.593	8.561
4.0	29.5	12.23	8.23	8.975	8.743	8.702
4.0	30.5	14.46	10.46	9.225	9.002	8.984
4.1	28.5	12.85	8.75	8.750	8.467	8.450
4.15	28.75	12.21	8.06	8.825	8.554	8.535
4.2	28.5	12.20	7.995	8.775	8.491	8.479
4.2	28.5	12.34	8.14	8.775	8.499	8.477
4.2	28.5	12.50	8.30	8.775	8.499	8.479
4.2	29.5	12.72	8.52	9.025	8.790	8.761
4.2	30.0	13.10	8.90	9.150	8.940	8.902
4.25	28.5	12.50	8.25	8.788	8.503	8.494
4.4	28.5	12.425	8.025	8.825	8.539	8.539
4.5	29.0	12.91	8.41	8.975	8.712	8.709
4.6	30.5	14.44	9.84	9.375	9.184	9.162
4.6	30.0	14.50	9.90	9.250	9.035	9.021
4.6	28.0	12.79	8.19	9.750	8.437	8.457
4.7	34.5	14.40	9.70	10.400	10.404	10.320

Contd....

1	2	3	4	5	6	7
4.7	28.5	13.10	8.40	8.900	8.611	8.630
4.8	29.0	13.46	8.66	9.050	8.783	8.799
4.8	27.0	11.91	7.11	8.550	8.185	8.235
4.85	29.0	13.31	8.46	9.068	8.795	8.817
4.9	28.0	13.82	8.92	8.825	8.508	8.546
4.9	26.5	12.82	7.92	8.450	8.060	8.123
5.0	30.0	14.10	9.10	9.350	9.130	9.140
5.0	30.0	14.23	9.23	9.350	9.130	9.140
5.0	28.5	13.58	8.58	8.980	8.682	8.717
5.05	30.0	14.89	9.84	9.363	9.142	9.155
5.2	27.5	13.74	8.540	8.775	8.430	8.494
5.2	28.75	14.25	9.05	9.087	8.804	8.847
5.3	37.25	13.71	8.41	8.738	8.379	8.453
5.4	27.50	13.99	8.59	8.825	8.478	8.553
5.6	29.5	14.74	8.47	9.375	9.123	9.177
5.6	28.25	14.58	8.981	9.063	8.750	8.825
5.7	26.5	14.39	8.69	8.750	8.250	8.361
5.7	28.5	14.13	8.44	9.150	8.848	8.925
5.9	26.5	14.55	8.66	8.700	8.298	8.420
5.9	27.5	14.50	8.60	8.950	8.597	8.702
6.0	32.25	16.48	10.48	10.163	10.041	10.072
		For prepared milk samples				
1.35	34.25	9.73	8.38	9.500	8.660	9.254
1.7	30.50	9.85	8.15	8.750	9.597	8.301

Contd....

1	2	3	4	5	6	7
1.7	30.50	10.14	8.44	8.750	9.597	8.301
1.7	31.0	10.19	8.49	8.775	9.008	8.442
2.2	30.0	10.48	8.28	8.650	7.890	8.308
2.4	34.0	11.90	9.50	9.700	8.824	9.496
2.5	33.0	11.72	9.22	9.475	8.622	9.244
2.7	29.5	11.10	8.40	8.550	7.883	8.316
2.7	30.0	11.07	8.37	8.775	7.994	8.457
2.7	32.0	12.19	9.49	9.275	8.440	9.021
2.85	29.5	11.99	8.14	8.688	7.914	8.360
2.9	34.25	12.47	9.56	9.888	8.984	9.715
2.9	32.5	11.36	8.46	9.450	8.594	9.221
3.3	28.0	11.62	8.32	8.425	7.674	8.071
3.55	32.0	12.45	8.90	9.488	8.618	9.273
3.55	30.0	12.31	8.76	8.988	8.172	8.709
3.6	30.5	12.78	9.18	9.125	8.294	8.865
5.7	29.25	15.12	9.42	9.338	9.454	9.136
6.1	28.0	14.48	8.38	9.125	8.259	8.903
6.8	27.0	16.10	9.30	9.050	8.182	8.829
7.0	30.0	17.01	10.01	9.850	8.893	9.734
7.6	28.5	18.22	10.62	9.625	8.684	9.489
8.3	28.0	17.88	9.58	9.675	8.719	9.556
8.5	28.0	17.39	8.89	9.725	8.761	9.128
8.7	24.75	17.28	8.58	8.963	8.078	8.758
8.9	28.0	18.68	9.78	9.825	8.844	9.734

Contd....

1	2	3	4	5	6	7
9.0	28.25	18.71	9.71	9.925	8.921	9.835
9.2	27.0	19.05	9.85	9.650	8.684	9.541
9.4	27.5	17.02	7.62	9.825	8.837	9.742
9.5	26.5	19.27	9.77	9.600	8.635	9.490
9.6	26.5	19.74	10.14	9.625	8.655	9.519
9.8	31.5	20.71	10.91	10.325	9.813	10.989
9.9	26.5	18.95	9.05	9.500	8.719	9.608
10.2	23.50	18.51	8.31	9.025	8.113	8.851
10.3	26.0	19.20	8.90	9.675	8.691	9.566
10.4	23.0	19.20	8.80	8.950	8.043	8.770
10.6	23.0	19.56	8.96	9.000	8.084	8.829
11.2	22.5	20.05	8.85	9.025	8.098	8.866
		For standardised milk samples				
3.4	30.75	12.37	8.97	9.158		8.876
3.5	30.5	12.36	8.86	9.100		8.836
3.6	30.0	11.64	8.04	9.000		8.724
3.6	30.5	12.78	9.18	9.125		8.865
3.6	30.0	12.34	8.74	9.000		8.724
3.7	30.75	12.49	8.79	9.213		8.965
3.7	30.0	11.53	7.83	9.025		8.754
3.7	30.5	12.38	8.68	9.150		8.895
3.7	30.5	12.37	8.68	9.150		8.895

VERIFIED Manjeet Singh Signature

D.A. 151662 28/1/83