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Review on Reference & Routine Measurement Techniques of Milk Fat based on Process, Cost and Instrumentation

Pankti Desai[1*] and Utpal Pandya[2]
[1]Research scholar, Gujarat Technological University, Gujarat, India
Email: panktidesai1@gmail.com
[2]Dept. of Instrumentation and Control, Sarvajanik college of Engineering & Technology, Surat, Gujarat, India
Email: utpal.pandya@scet.ac.in

***Abstract*—Milk is arguably natural product which contains a number of components of nutritional significance. Milk fat is an important measure of its quality because of salubrious and technological aspect of its industrial use in interest of human. This makes determination of milk fat essential. This paper gives overview of routine and reference methods used and recent trends of fat measurement in dairy industries. Comparative study of technical and commercial aspects of different methods gives the idea of its distinctive uses. Comparison of Optical techniques based on UV, VIS, and IR wavelengths give distinct analysis, also pros and cons of analytical measurement of process. Detail analysis manifests scope of improvement which gives aspiration to develop low-cost automated reference technique for milk fat.**

***Index Terms*— Reference method, Gerber Test, Butyrometer, Routine method, Spectroscopic techniques and MilkoScan.**

I. INTRODUCTION

Quality of milk plays an important role in human growth. Composition of milk defines its quality and affects its attribute. Milk fat is an important source of energy, especially for a growing and developing organism. It is comprising of triacylglycerols (TAGs), diacylglycerols, phospholipids, fatty acids, and cholesterol (Jensen, 2002). Each of these components of milk fat pays important role in providing energy source when they are metabolized in the human body. The TAGs are major integral and defining about 97 – 98 % of the total milk fat content (Vieiteza, Irigaraya, Callejasa, & et.al, 2016). Milk fat being most variable component depend on ruminant milk (Samková, Spicka, & et.al, December 2011),as per the requirement of variety fat milk, raw milk is converted in accordance with different fat and milk solid-not fat (SNF) content (the Food Safety and Standards (Food Products Standards and Food Additives) Regulations, 2011). Milk pricing is done based on its fat which affects its physical, chemical and nutrition properties. Examination of Fat and SNF of milk decides its price (Anon., 2019).Table I (Anon., 2017). shows the basic & standard techniques described by the international standards. There are mainly two types methods Reference and Routine. This paper gives complete overview of techniques used for milk fat measured and a comparison based on their methodology, sensing technology, Unique requirement of apparatus, its applications for different dairy products and their scope of improvement. Reference methods are offline chemical methods used to analyze fat, protein etc., whereas routine methods are

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at-line, optical methods use to find detail milk properties along with basic elements (https://www.icar.org, 2017). In the paper section 2 reference method is discussed and section 3 gives overview of routine methods. The latest updates on recent machines used by dairy industry are inked in succeeding part of paper.

II. Reference Methods

Reference methods are rigorously studied and internationally standardized methods (i.e., ISO, IDF, AOAC methods) (Milk — Determination of fat content — Gerber butyrometers ISO 488:2008(en), 2008-09; https://www.icar.org, 2017). These methods are used to evaluate the reliability and calibration of other measurement procedures. Having the highest metrological properties these are the primary methods when applied correctly provides traceability to the SI system of units (Milton & Quinn, 2001). The reference methods are performed with specific instruments which generates "Attributing values" for other techniques, machines and for other laboratories if it's part of system with centralized calibration. Routine methods have major contribution in Centralized calibration concept. (https://www.icar.org, 2017).

TABLE I. BASIC METHODS OF MILK FAT MEASUREMENT (HTTPS://WWW.ICAR.ORG, 2017)

Milk Fat Measurement Techniques	Refrence methods	Primary international reference methods Rose-Gottlieb (ISO 1211 | IDF 1)
		Secondary international reference methods -Gerber(ISO 19662 | IDF 238) -Babcock(AOAC 989.04)
	Routine Method	Standardized routine methods -Automated turbidimetry AOAC 969.16 -Mid infrared (MIR) spectrometry ISO 9622 | IDF 141

III. Type of Reference Method

A. Gerber method

The Swiss chemist and dairy-owner Niklaus Gerber developed the Gerber method (Badertscher, Berger, & .Kuhn, 2007). It is an elementary rapid, and economical method for determination of the fat content of various milk and milk products and popularly adopted by Indian and International dairy industries (Indian Standard Apparatus For Determination Of Milk FAT by Gerber Method - Specification,IS 1223 : 2001:i, 2001).This method is famous worldwide in a various application like payment testing and process standardization. (DIick., Joanna.Ynch, & at.al, 2001). This technique is unique due to its requirement of several specialized apparatus like butyrometers, pipette, centrifuge, and water bath, to carry out the test (James, 1995). The special Gerber centrifuge, Butyrometer are shown in (indiandairys.blogspot.com, n.d.) Fig 1 (a) and (b) respectively. In Procedure of a test, portion of milk is pipetted into a Gerber milk butyrometer containing sulfuric acid. Isoamyl alcoholic added, and the contents of the butyrometer are mixed to dissolve the milk and release the fat. The released fat is isolated in the neck of the butyrometer by centrifugation as shown in Figure 1c. The percentage of fat in milk (g/100 g) is determined by reading the calibrated scale on the neck of the butyrometer (Badertscher, Berger, & .Kuhn, 2007; DIick., Joanna.Ynch, & at.al, 2001; Milk — Determination of fat content — Gerber butyrometers ISO 488:2008(en), 2008-09).It has been observed that to improve accuracy of the Gerber test the calibration of the butyrometer must be unchanged, so that it may be considered as an international instrument, while the capacity of the milk pipette may be adapted to national or regional needs provided its volume. (W.Crocker, D.Jenkins, & at.al, 1955). This method due to its inexpensive and rapid process result is famous in India and Europe's dairy industry. Different butyrometer are designed for different dairy products such as whole milk, skim milk, and cream. (Xiong, B.Adhikari, & at.al, 2016). Due to use of concentrated sulfuric acid, it is difficult to automate the Gerber test. The handling of butyrometer requires practical experience, which has an adverse effect on the robustness of the method (Habib, 2014).Many experiments are done to establish relation between the fixed butyrometer scale with the fat values. (F.DAY & Grimes, 1918).

(a) (b) (c)

Fig 1.Special equipment used in Gerber Test (a)Gerber centrifuge (b)Butyrometer (c)Butyrometer with extracted milk fat after centrifugation. (indiandairys.blogspot.com, n.d.)

B. Rose-gottlieb method (Mojonnier method)

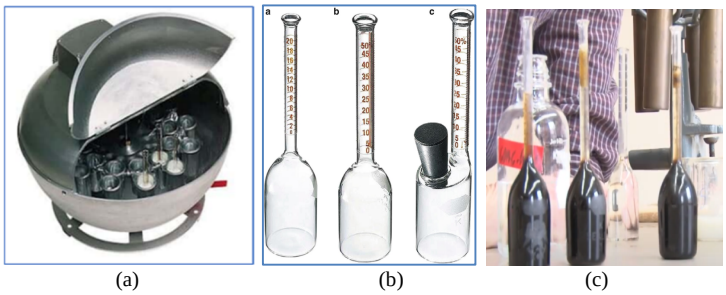
Extraction of fat which was not available in Gerber was modified by the method. The milk sample is mixed with ammonia and ethyl alcohol; which dissolve the protein and the latter to help precipitate the proteins. Fat is extracted with diethyl ether and petroleum ether. Special instrument called Mojonnier fat extraction flask plays important role. Mojonnier centrifuge, Mojonnier Fat Extraction Flasks shown in Fig 2. a, b respectively and c shows the Mojonnier flask with extracted milk fat after centrifugation. After evaporation of Mixed ethers remaining residue is weighted. This method is considered suitable for reference purposes (Sectional, 2009; Anon., 2016).

(a) (b) (c)

Fig 2.Special equipment's used in Rose-gottlieb method. (a)Mojonnier centrifuge(b) Mojonnier Fat Extraction Flasks(c)Mojonnier flask extracted milk fat after centrifugation. (Flohr Instruments, n.d.)

C. The Babcock Method

Similar to Gerber method here concentrated sulphuric acid (92% or 1.82 specific gravity) is added to 18 grams of milk and fat is separated by centrifuge which acidified sample at 50°C improved the separation as the fat was deaerated, it floated and its volume could be measured (Herreid & O.Ernest, April 1942; Sectional, 2009) . A specific Babcock graduated test tube as shown in Fig 3c was designed for the method and the amount of fat quoted as %w/w milk. The whole test is of about 45 minutes. Fig 3 gives idea of special Babcock centrifuge, Babcock graduated tubes and its look after fat extraction.



(a) (b) (c)

Fig 3Special equipment's used in Babcock method. (a)Babcock centrifuge(b) Babcock tube(c)Babcock tube after extracted milk fat after centrifugation. (Flohr Instruments, n.d.)

D. Comparison of Basic refence method

The reference methods are predominant either it may be Gerber, Babcock or Rose-Gottlieb. Babcock method is limited to raw milk testing whereas Gerber test is applicable to pasteurized homogenized milk (DIick., Joanna.Ynch, & at.al, 2001). Gerber's high degree of accuracy and reproducibility compared to the Mojonnier

ether extraction test. (weberscientific.). Babcock test on nonhomogenized milk slightly higher than the Mojonnier, Gerber test is by all odds the most satisfactory test studied for making fat tests of homogenized milk (Lucas, G.Trout, & at.al, 1974) . Babcock method has Limitation that phospholipids are not determined and it is not suitable for products containing chocolate or added sugar because of the extreme damage done to these compounds by high acid concentration (Otieno, 2019). These methods provide accurate results, which matches with the internal SOP according to the ISO International Standards. By contrast, reference methods have several disadvantages – time-consuming process of analyses and fewer analyzed samples. (Badertscher, Berger, & .Kuhn, 2007). Also reference method has limitation to identify limited properties of milk (eg.fat, protein, lactose). It cannot further more find concentration of particles of fats like triacylglycerols (TAGs), diacylglycerols, phospholipids, free fatty acids etc. Table II shows the comparison of above said methods based on their accuracy, performance time and specific requirements.

III. ROUTINE METHODS

Reference methods like Gerber, Rose-Gottlieb are time consuming and require special chemicals and specific lab equipment. Therefore, methods which allow simple, ideally non-destructive and accurate analysis need to be developed and improved (Vaskova, Buckova, & et.al, 2016).New techniques with features like small portable measuring unit and compatible to connect with automatic control units are prefer. Routine methods are developed by measuring different properties of milk and calibrated in terms of fat. Major routine methods are based on optical properties of milk. In Present day, trend is to develop rapid, automatic and cost-effective analytical methods which establish identification of components with various physico-chemical properties in a single analytical run (Biziuk, Tankiewicz, & at.al, 2018).In this perspective new spectrometry methods (UV- visible, infrared, luminescence or Raman spectroscopy) are reassuring techniques. Spectroscopy pertains to the dispersion of an object's light into its component colors (i.e. energies). By dissection and analysis of an object's light, one can infer the physical properties of that object like temperature, mass, luminosity and composition. Different wavelengths of light manifest themselves as different colors.

TABLE II. COMPARISON OF DIFFERENT ROUTINE METHO

Method	**Material used**	**Sensor/Instrument used**	**Fat separation Time (Process time after sample preparation)**	**Accuracy**	**COST(LEAST)**	**Application**	**Comments**	**Measurement method**
Gerber	Sulphuric acid (sp.gr.1.82), Amyl alcohol (sp.gr.0.82-0.83)	Butyrometer, milk pipet, Gerber centrifuge	10-15min	≥Rose-gottlie	15000	Best for homogenized milk, also applicable milk, cream and frozen desserts etc	Butyrometer reading and fat calculation require skill, due to chemical process cannot automated.	Manual
Röse-Gottlie	Ammonia and ethyl alcohol, diethyl ether, petroleum ether	Mojonnier fat extraction flask, Mojonni centrifuge	30 min	>Babcock method	25000	Cream, non-homogenized milk and other milk product.	Not applicable for homogenized milk, Mojonnier handling require skill,	Manual
Babcock	sulphuric acid (92% or 1.82 specific gravity	Babcock graduated test tube, Babcock centrifuge	45 min	<Gerber, rose-gottlie	30000	Applicable to find butterfat in all milk.	Time consuming, not applicable for ice-cream, low accuracy	Manual

The energy of a light wave is inversely-proportional to its wavelength (Kulesa, 1997). Several regions of the electromagnetic spectrum with their wavelength and wavenumber limits for different spectroscopic techniques are describe in Fig 4.

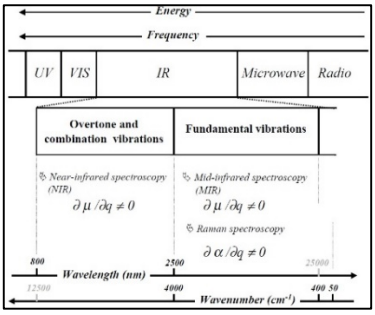
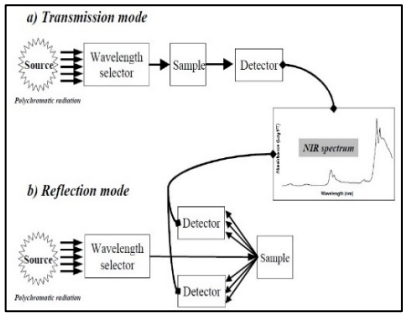
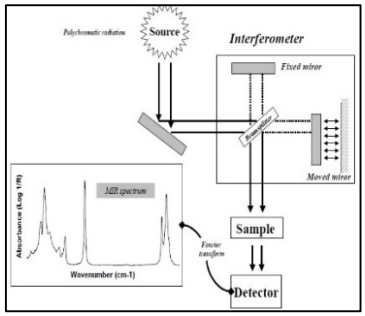


Fig 4 Regions of the electromagnetic spectrum and the related (Dardenne, Baeten, & at.al, 2002)



(a)
Fig 5 .(a). Schematic configuration of a dispersive NIR instrument;



(b)
Fig.5 (b) Schematic configuration of an FT-MIR instrument. (Dardenne, et al., 2002)

The review of routine method is done based on UV, VIS, and IR wavelength range. The milk is composed of water (87%), fat (4%), protein (3.4%), lactose (4.8%) and other elements (0.8%), such as acid citric and minerals (https://www.groupe-esa.com/ladmec/bricks_modules/brick02/co/ZBO_Brick02_2.html). The optical light–milk relations is obtained by the correlation between the size of particles suspended and the wavelength of incident light (Aernouts, Beers, & at.al, 2015). Dependency of the amount of light scattered by the fat globules when milk is diluted with a protein-dispersing reagent such as alkaline versine (EDTA) depends upon two variable one fat content and other is the size distribution of fat globules (Michalski, Gassi, & at.al, 2003). Standardized routine method for measurement of fat as per ICAR is Automated turbidimetry method (https://www.icar.org, 2017). The turbidic analysis measures the absorbance or per cent transmission. It was established by the observation of correlation between fat content and the detected elimination of light dispersed by a milk sample at specific wavelengths (U.S.Ashworth, February 1969,).This method's vulnerability is high to the size variability of colloidal milk particles, even in homogenized milk, and thus it is now outworn (SergeyKucheryavski & AnastasiiaMelenteva, 2014). Major application of this method is for drinking milk (whole, semi-skimmed and skimmed milk) although it had good results with raw milk samples too (O.Hanuš, J.Říha, & et.al, 2014). The next popular routine method is the Ultraviolet absorption spectrophotometric which was developed to measure fat from absorbance at 400 mμ with a round cuvette. This method does not require prehomogenization milk like turbidity measurement, because of a dispersing effect by the combination of reagents (LE & et.al, 1970). UV-spectrophotometry is best fit due to a fleet, invaluable alternative screening method to estimate milk fat content in small samples without prior lipid extraction (Forcato, M.Carmine, & et.al, 2005). These optical methods are based on principal of scattering, absorption and reflection of light through milk sample and based on principal optical sensor are selected which work on principal of intensity modulation. Spectroscopic methods use absorption principal to determine fat (Lamorska & et.al, July 31st 2013) . Several milk analysis techniques are separated according to the light wavelength spectra operation that are visible (VIS), short-wave (SWIR), near (NIR), mid (MIR), long-wave (LWIR), or thermal infrared (TIR) light (Meer, 2018). Based on scattering principal simple and rapid fat and protein content in milk using laser beam was developed by Qi Xin (Qi.Xin, Ling, & et.al, 2006). laser beam at 632.8nm was used for sample which was homogenized and was prepared by adding chemical reagents to dilute milk. The average diameter of the fat and protein in sample 2000nm and 120 nm respectively. Other particles (the lactose, the inorganic salt, etc.) get dissolve in the milk, causing their diameters are much less than that of the fat and the protein. As per Mie theory only the biomolecules can cause obvious light scattering, scattering of the other particles can be ignored. New Technique based on diffusion spectroscopy analysis in the Vis and adjacent near infrared (NIR) spectroscopy region (400-995 nm) was developed suited for in-line process monitoring and field analysis in dairy industry. Fig.5a show the configuration of NIR spectroscopy. Distinct spectral component for various sized fat globules were adequate for their quantitative analysis using multivariate modelling like partial least-squares (PLS) regression (A.Bogomolov, V.Belikova, & et.al, 2017). Another NIR spectroscopic for measurement of milk components makes used region from 1100 to 2500 nm using several partial least squares calibration models (A.Kalinin, V.Krivtsun, & et.al, 2008).ATR-FTIR spectroscopy with spectral range for NIR region from 750 to 2500 (13400 – 4000 cm^{-1}), while for MIR it is between 2500 and 25000 nm(4000 – 400 cm^{-1}) along with was with chemometrics was used for detection of presence of pig body fat in pure ghee (N.Upadhyay, P.Jaiswal, & et.al, 2018).Fig 5b .gives idea of functionality of MIR system. All routine method compares the result with the result of reference methods for example Mlček et al. (Mlcek J, 2016) determined milkfat using NIR which uses Gerber and Röse-Gottlieb reference methods for calibration.

Fourier Transform Infrared (FTIR) is a spectroscopic technique that uses electromagnetic spectrum of wavelengths between 2,500nm and 25,000nm. Natural raw milk has a particular infrared spectrum. With the help of FTIR analysis, it is possible to program an Analyzer to identify the spectra representing pure raw milk. (Mills, 2016).In FTIR technique, IR radiation is absorbed when passed through sample which cause the changes in the variation of the dipole moment of molecule due to which its rotational-vibrational energy changes. This method is applicable to raw and skim milk, cream and other dairy products. Similarly, Raman spectroscopy provides very specific chemical "fingerprint" of every single chemical substance in the form of the Raman spectrum (Dardenne, Baeten, & at.al, 2002). Determination of fat depends on the changes in the relative Raman band intensities correlated with the overall change in milk fat content (R.El-Abassy, P.Eravuchira, & et.al, 2011). Fig 6 is schematic configuration diagram of an FT-Raman instrument. In overall research comparing results of different spectroscopic technique used in milk fat measurement with refence methods, In mid-range spectrum IR spectroscopy with filter technology in mid-range of spectrum & IR spectroscopy with whole spectrum and FT in mid-range of spectrum has most impressive results.

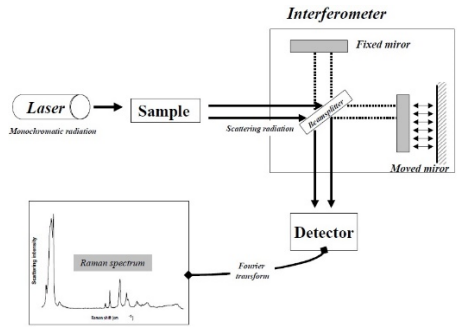


Fig 6.Schematic configuration of an FT-Raman instrument (Dardenne, Baeten, & at.al, 2002)

Fig 7.MilkoScan™ FT+ Latest Device used for analysis of Milk in Dairy Industry *(FOSS)*

NIR and turbidimetric method and ultrasound method are in succeeding order respectively.IR technologies are the highest expensive technology for implementation in a dedicated system (BNER & et.al, 1997). NIR technology have special requirement of specialized light source, wavelength detector and specialized sophisticated processing software. These requirement of specific hardware and software costs for detail analysis which could be more than the whole milk production system. Comparison of different routine methods for analysis of different milk properties are discussed in Table III.

TABLE III. COMPARISON OF DIFFERENT ROUTINE METHODS FOR DIFFERENT MILK PROPERTIES MEASUREMENT

Method	**Principal**	**Wave length**	**Component Determination**	**Accuracy for Fat**	**Process time**	**Cost**
Turbidimetric analysis	Absorbance	0 to 4,000nm	Fat, protein	0.994	<10 Min	Rs.30-40 k
Ultrasound	Absorbance	100-400 nm	Fat, protein	0.995	<10 Min	Rs 47250- Rs 550000
Visible spectroscopy laser	Scattered-transmitted	632.8 nm	Fat, protein, Lactose	0.973	<10 Min	Rs 155000/number
NIR	Reflectance	400-995 nm	Fat, protein, Lactose	0.952	<10 Min	Approx. Rs 15-20 Lac
SWNIR	Transmittance	700–1100 nm	Fat, protein, Lactose	0.996	<10 Min	Approx. Rs 15-20 Lac
FTIR	Absorbance	2,500nm and 25,000nm	Fat, protein, Lactose	0.9	<5 Min	Rs.50 to 80 Lac
FT-Raman	Absorbance	900−3400 cm^{-1}	Fat, protein, Lactose	0.99	<5 Min	Rs.50 to 80 Lac

Above mentioned Routine methods are suited for high-capacity milk/ dairy industry, for small scale dairy industry low-cost techniques having low power consumption is preferred. Instead of high machine and software low-cost embedded systems like microcontrollers, microprocessors, and field-programmable gate arrays (FPGA), based system can be productive. Several attempts have been made to develop embedded sensing systems based on microcontrollers can be observed for Parametric Data Monitoring & Quality Analysis of Milk (Khajwal & et.al, 2019),ARM controller was tried to measure milk constituents (Z. Zhou & et.al, 2006). Also, different try had been

done for automatic sensing system to detect 'synthetic milk' or milk adulteration (S.Das, B.MulintiSivaramakrishna, & et.al, 2015). Embedded system can be enhanced by including like wireless communication, internet of things (IoT), dynamics databases which can help in accessing the information from any access to the internet from worldwide. Milk monitoring and milk adulteration system with IoT technology are now a days in trending (G.Rajakumar, Kumar, & et.al, 2018).Problem related to such system is matching the accuracy with high end optical spectroscopy techniques. Indian local village milk vendors and small dairy industry use Gerber reference technique. Difficulty faced by them is special skill requirement to handle chemicals and butyrometer reading. Also, bigger Dairy industries like Amul Ltd, Sumul Ltd (Gujarat, India) consider Gerber test for reference method for detection of fat for different dairy products along with latest routine methods. Different routine and reference methods are compared in Table IV based on accuracy, Process time and cost. The ongoing trend in routine method emphasize on the development of optical techniques. In order to obtain online systems to predict milk composition onsite, without the use of chemical reagents, in a fraction of the time compared to laboratory tests.

TABLE IV. COMPARISON OF REFERENCE & ROUTINE METHOD

Method	**Principal of Detection**	**Accuracy**	**Process time**	**Component Determination**	**cost**	**Automation/Automatic Readings**
Reference	Chemical	Higher compare to Routine (average 0.01)	Higher than Routine method	fat	low cost	Manual
Routine	Optical	Calibration of device is done with reference methods (Average 0.9)	Less than Reference	fat, Protein, Lacoz	high c ost	fully automatic

IV. LATEST TRENDS IN MILK FAT MEASUREMENT IN DAIRY INDUSTRY

Recent trends in Industrial laboratories apparatuses based on Fourier transform infrared technology (The MilkoScan FT 6000 (Foss Electric)) are used which provide compositional parameters including fat, protein, lactose, solids, CN, FFA, urea, and FP depression. (A.Sa´nchez, Sierra, & et.al, 2007).It has a high capacity (up to 600 samples per hour) FTIR technology which provides potential for analysis of all component in milk. Self-cleaning technique of the MilkoScan FT+ ensures high up-time and optimal performance. Machine has advance data output interface facilities like Real-time display & print-out, storage on hard disk, RS232 and PC network transmission (TCP/IP) and Data export using CSV files, CS83 protocol or XML (FOSS). Fig 7 give visualization of MilkoScan FT machine.

V. CONCLUSION

It can be ascertained that recent current trend is in the development of optical techniques to get online rapid determination of milk composites. The potential of routine spectroscopy techniques are widely used with its mortal use of on-line systems, but still remains expensive. The best techniques are NMR, but this could only be used as an offline technique because of its cost. Reference methods on other hand are low cost compare to routine methods but have disadvantage of handling limited number of samples and missing automation and online measurement. And In Practice the routine methods are always compared with reference methods According to market scenario reference methods are used by milk vendors of villages and also by big dairy industry for standardization. Result of Routine methods are accurate and has constitutive part for examination in quality of milk and dairy products but not affordable by all milk suppliers and small-scale dairy industries. Developing country like India Gerber test is most popular reference method. The major hurdle in automation of Gerber test is understanding of special instrument (butyrometer) design, its complex relation between reading scale and milk fat. This requires detail study and research on redesign scope of butyrometer to match recent requirement of automation. The modernistic approach presented in the literature results show a valuable effort for selection of the best technique for rapid and precise estimation and consequently, the development of online systems.

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