Evaluation of Methods for Estimation of Sucrose in Selected Milk based Sweets

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE
(DEEMED UNIVERSITY)

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN

DAIRY CHEMISTRY

By

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B. Tech. (Dairy Technology)

DAIRY CHEMISTRY SECTION
NATIONAL DAIRY RESEARCH INSTITUTE (ICAR)
BANGALORE- 560 030, INDIA

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DEDICATED

TO

MY BELOVED FAMILY

EVALUATION OF METHODS FOR ESTIMATION OF SUCROSE IN SELECTED MILK BASED SWEETS

By

MADHUMITA MAJUMDAR

Thesis Submitted to the

National Dairy Research Institute

(DEEMED UNIVERSITY), Karnal

In partial fulfillment of the requirement for the award of the degree of

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IN

DAIRY CHEMISTRY

Approved by

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CERTIFICATE

This is to certify that the thesis entitled, "**EVALUATION OF METHODS FOR ESTIMATION OF SUCROSE IN SELECTED MILK BASED SWEETS",** submitted by **Ms. MADHUMITA MAJUMDAR** towards the partial fulfillment for the award of the degree of **MASTER OF TECHNOLOGY** in **DAIRY CHEMISTRY** of the **NATIONAL DAIRY RESEARCH INSTITUTE (Deemed University),** Karnal (Haryana), India, is a bonafide research work carried out by her under my guidance, and no part of the thesis has been submitted for any other degree or diploma.

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Date: 11th June 2012 *Madhumita Majumdar*

ABSTRACT

In our country, a number of delicious milk sweets such as *gulab jamun, burfi, peda, kheer, payasam, halwas* are popular which use milk solids, sugar and starch in the form of *maida* or *suji* for their preparation. Estimation of sucrose in these products is necessary for checking complete compliance to standards and to meet labeling pre-requisites. However, the presence of lactose and starch complicates the estimation of sugar in these products. In the present study, Lane-Eynon, Munson-Walker-Bertrand, polarimetric and Seliwanoff's colorimetric methods were evaluated for the estimation of sugar in *gulab jamun* and *chhana podo* both containing lactose, sucrose and starch as the source of carbohydrates. Among the four methods, Seliwanoff's method appeared to be accurate for estimation of sucrose. The presence of starch and its inversion products could be responsible for the inadequacy in the determination of sucrose by the other three methods. Hence, attempts were made to optimize Seliwanoff's colorimetric method to estimate sucrose in these products. A standard solution containing sucrose, lactose and starch in a ratio of 3:1:2 was used for the optimization. An aliquot of the solution added with 2 ml of 0.1% aqueous resorcinol solution and 6 ml of concentrated hydrochloric acid was incubated at 70^{o}C (optimum temperature) for periods ranging from 10 to 40 minutes with an increment of 5 min. The developed colour was then measured for absorbance at 490 nm in a spectrophotometer. The sucrose level was estimated using a standard curve. It was observed that an incubation time of 30 min was optimal and the estimated sucrose level was within ±1% of the actual level. The method was tested with *gulab jamun* and *chhana podo* containing varying levels of added sucrose/ sugar. *Gulab jamun* and *chhana podo* without added sugar did not show any sucrose level. Analysis of *gulab jamun* samples spiked with 20, 25 and 30% sucrose and 20, 25, 30, 35, 40, 45 and 50% cane sugar and *chhana podo* with 20, 30 and 40% sugar showed that sucrose level could be estimated with an accuracy of ±3% by the colorimetric method. The sugar content in market samples of *gulab jamun* and *chhana podo* estimated by the optimized method was 38-44 and 24.4-24.8%, respectively. The method was also used for estimation of sucrose in dietetic sweets and the values matched with the level of sugar present.

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LIST OF ABBREVIATIONS

%	= Percentage
^{o}C	= Degree centigrade
ANOVA	= Analysis of variance
cm	= centimeter
DB	= Dry basis
dcm	= decimeter
g	= gram
h	= Hour
in	= Inches
kg	= kilogram
max.	= maximum
mg	= milligram
min	= Minute
min.	= minimum
ml	= milliliter
mm	= millimeter
N	= Normal
nm	= nanometer
SE	= Standard error
sp. gr.	= Specific gravity
UV	= ultra violet
WB	= Wet basis
wt	= weight

Chapter- 1

Introduction

1.0 INTRODUCTION

In our country, a number of delicious milk sweets such as *gulab jamun, burfi, peda, kheer, payasam, halwas* are available which use milk solids, sugar and starch in the form of *maida* or *suji*. Some of these sweets also contain ingredients such as nuts, raisins and added colours.

Preparation of many Indian sweets is time consuming and is a job that involves art and science. It is evident from the historical evidences that the art of preparing sweets from surplus milk was developed centuries ago. In the present era, in addition to religious and social needs, the milk sweets are prepared for value addition. That is why, now their manufacture is not confined to only small confectioners (*halwais*), but many organized dairies and large players in the milk business have entered into this lucrative venture.

There is a lot of variation in the types and levels of ingredients used and methods of preparation which result in a wide variation in the physico-chemical and sensory quality of the milk sweets. In this regard, variation in sugar level appears to be the highest. However, there are no legal or voluntary standards for most of the traditional Indian milk sweets. BIS standard for sugar level in *rasogolla* is 45% (max.) (IS: 4079-1967) while for *burfi* and *mawa burfi* it is 40 and 48% (max. by percentage wt.), respectively (IS: 5550-1987).

The sugar content of the product is often calculated based on the level of addition during preparation and the yield of the final product. This may not give correct information on the level of sucrose in the product. In the conventional methods (Lane-Eynon, Munson- Walker or polarimetric method) for determination of sucrose in milk and milk products, inversion of sucrose is done and then the difference in either reducing property or optical activity is measured as compared to lactose. But these methods may not give accurate results for several milk based Indian sweets which contain starch along with lactose and a high level of sucrose. Starch is added as a binder (e.g., *gulab jamun, burfi* etc.) or for giving its special aesthetic value (*gujia, gaja, labangalatika* etc.) or may be present as an important ingredient (*halwa, malpua*, *kheer/ payasam,bengali pitha*s etc.). When

tested by these methods, some of the starch also may get hydrolyzed during inversion leading to erroneous result on sugar level. Hence, these methods need to be evaluated for their efficiency for sugar estimation in milk products containing starch. Performance of modified Seliwanoff's method as used for estimation of sugar in ice cream and sweetened condensed milk also needs to be evaluated for its usefulness in milk sweets.

Estimation of sugar content in food products becomes necessary in today's era of worldwide marketing where many attempts are being made to introduce the traditional Indian sweets in the global market. This, in turn, requires a strict quality monitoring procedure prior to launch. The prerequisites for marketing food products are detailed composition on package, complete compliance to standards and comprehensive information on nutritional parameters on every viable unit. For each of these, determination of exact sucrose content is essential.

Moreover, in the recent times, dietetic milk sweets are commercially available with claims such as "Sugar Free", "Diabetic Sweets". However, whether these products are free from sucrose or not needs to be ascertained.

As there is an increasing demand for packaged/ canned, commercially viable sweets, the competition between different manufacturers is also escalating. To maintain a desired quality level, conformation to standards must be checked thoroughly and for that purpose, standardization of a method for estimation of sucrose content in milk-based sweets is of huge importance. In this regard, a study had been proposed with the following objectives:

- Evaluation of methods for estimation of sucrose (sugar) in *gulab jamun*, a *khoa* based product and *chhana podo*, a *chhana* based product.
- Standardization of the method(s) for the estimation of sucrose in *gulab jamun* and *chhana podo.*

Chapter- 2

Review of Literature

2.0 REVIEW OF LITERATURE

The prevailing milk sweets in Indian market can be broadly classified into two groups, viz. *khoa* based and c*hhana* based. In terms of regional preferences, the *khoa*-based sweets are more popular in northern, western and southern parts of the country while *chhana*-based sweets are popular in eastern India.

Gulab jamun, a *khoa* based sweet is highly popular in northern, western and central parts of India (Mathur, 1991). It has been traditionally prepared in small scale by local *halwai*s. However, in recent days, there is growing interest among the organized dairies to produce this sweet on commercial basis and a decisive lead was taken by NDDB in this direction (Aneja, 1991).

The gross chemical composition of *gulab jamun* varies widely depending on numerous factors, such as composition and quality of *khoa*, proportion of ingredients, sugar syrup concentration, etc. The composition of *gulab jamun*, on the drained weight basis, is given in Table 2.1.

Chhana podo is a traditional product of Orissa. It has a spongy, particulate and chewy texture with compact to grainy body with visible inter particle spaces and a typical cooked, sweet and nutty flavour (Ghosh *et al.*, 2007). Two types of *chhana podo* viz. dry and wet type, have been reported (Ghosh *et al.*, 2002). The composition of both the types is listed in Table 2.2.

Estimation of sugar content in food products becomes necessary in today's era of worldwide marketing where many attempts are being made to introduce such indigenous sweets in the global village. This, in turn, requires a strict quality monitoring procedure prior to launch. Estimation of sucrose in these products is also necessary for checking complete compliance to standards and to meet labeling pre-requisites.

Table 2.1: Gross chemical composition of *gulab jamun* (Drained weight basis)

Constituent	Per cent
Fat	10.0
Proteins	6.0
Sugar	42.0
Other solids	14

(Aneja *et al.*, 2002)

Table 2.2: Gross chemical composition of *chhana podo*

Constituent	Dry type	Wet type
	Range (Per cent)	
Moisture	29-35	40-50
Fat	21-23	16-22
Protein	15-17	10-17
Sugar	18-21	15-24
Starch	6-8	5-11

(Ghosh *et al.*, 2002)

2.1 Estimation of carbohydrates

2.1.1 Total Carbohydrates

Total carbohydrate content of foods has, for many years, been calculated by difference, rather than analysed directly. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed

and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula:

100 - (weight in grams of protein + fat + water + ash + alcohol in 100 g of food)

It should be clear that carbohydrate estimated in this fashion includes fibre, as well as some components that are not strictly speaking carbohydrate, e.g. organic acids (Merrill and Watt, 1973). Total carbohydrate can also be calculated from the sum of the weights of individual carbohydrates and fibre after each has been directly analysed (FAO, 1998).

2.1.2 Lactose

Lactose is the major carbohydrate in milk. It generally ranges from 4.5-4.9 per cent. It is correlated with many physicochemical properties and various biochemical proceses of dairy products (Mathur *et al.,* 1999). Therefore, estimation of lactose is important and it can be quantified by different methods. The main five principles are:

- Oxidation- Reduction titration
- Polarimetry
- Colorimetry
- Enzymnatic
- Chromatography.

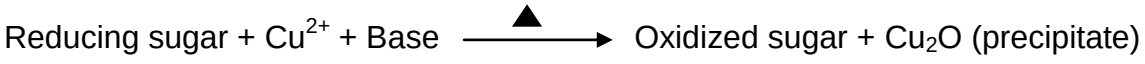
Most of the traditional methods are time consuming, as the quantitative determination of lactose can be performed only in the soluble serum samples obtained after prior removal of fat and protein fractions (Corzo *et al*., 2009).

2.1.2.1 OXIDATION- REDUCTION TITRATION

Methods following this principle are Lane-Eynon method, Munson Walker method and Chloramine- T method (Mathur *et al.,* 1999).

2.1.2.1.1 Lane-Eynon Method

This method principally involves quantitative reduction of alkaline cupric salts in Fehling's solution (copper sulphate in sodium potassium tartrate) to red cuprous oxide by titration with lactose, a reducing sugar, on boiling. Methylene blue is used as the redox indicator. From the amount of copper salt reduced, the quantity of lactose is calculated (Mathur *et al*., 1999).



Grimbleby (1956) reported that one of the most important stages governing the accuracy of lactose determination in milk was the preparation of the protein-free serum. Factors influencing the lactose content of the serum were:

(1) The complete precipitation of protein,

(2) The formation of lactose-protein complexes, and

(3) The degree of hydration of the protein coagulum.

Experiments had been described to show how these factors were influenced by different clearing agents operating at different pH values. On the basis of the results obtained, a reagent containing zinc acetate, phosphotungstic acid and acetic acid was suggested which reduced to a minimum any errors introduced by the above factors.

The disadvantages of this method are

(i) The results depend on the precise reaction times, temperatures and reagent concentrations used and so these parameters must be carefully controlled;

(ii) It cannot distinguish between different types of reducing sugars,

(iii) It cannot directly determine the concentration of non-reducing sugars, and

(iv) It is susceptible to interference from other types of molecules that act as reducing agents (Srivastava, 2010).

2.1.2.1.2 Munson Walker Method

Munson and Walker method is an example of a gravimetric method of determining the concentration of reducing sugars in a sample. Carbohydrates are oxidized in the presence of heat and an excess of copper sulfate and alkaline tartrate under carefully controlled conditions which leads to the formation of a copper oxide precipitate:

The amount of precipitate formed is directly related to the concentration of reducing sugars in the sample. The concentration of precipitate present can be determined gravimetrically i.e., by filtration, drying and weighing (Srivastava, 2010). Alternatively, the wet precipitate of cuprous oxide is dissolved in known qualtity of saturated solution of ferric ammonium sulphate. This solution is then titrated with the standard solution of potassium permanganate. As one milliliter of 0.1 N potassium permanganate is equivalent to 0.00635 g of copper, the weight of cuprous oxide in mg present in the known quantity of filtrate used can be determined from the titre value (Bertrand, 1906). However, the use of standard potassium permanganate solution for titrating the reduced cuprous oxide according to the Bertrand modification, had shown the disadvantages of instability of solution, sensitiveness to foreign organic matter and poor end-point, especially when the solutions cannot be thoroughly cleared (Wildman and Hansen, 1940). Weinmann (1944) modified this method by using 5 ml aliquots of the sample, which allowed amounts of glucose ranging from 0.1 mg to 20 mg in the test portion and estimated accurately to the nearest 0.1 mg.

Another approach is to add an excess amount of copper sulphate to the lactose containing solution. After cooling, the untreated copper sulphate is determined by the reaction with potassium iodide. The liberated iodine being titrated against standard sodium thiosulphate($Na_2S_2O_3$) solution using starch as an indicator.

$$2\ CuSO_4 + 4\ KI \longrightarrow CuI_2 + 2\ K_2SO_4 + I_2$$

$$I_2 + 2\ Na_2S_2O_3 \longrightarrow 2\ NaI + Na_2S_2O_6$$

Then the concentration of lactose can be calculated since the oxidation of one mole of lactose (360 g) yields one mole of cuprous oxide (143 g). Otherwise, the lactose equivalent to the quantity of oxide can be read from Munson-Walker/ AOAC Tables (Fox and McSweeny, 1998).

This method is an empirical one and suffers from the same disadvantages as the Lane-Eynon method; nevertheless, it is more reproducible and accurate (Srivastava, 2010).

2.1.2.1.3 Chloramine-T Method

Since the end point in the Lane- Eynon metod using Fehling's solution is not sharp, the redox determination of lactose can be done with chloramine-T (N-chloro- p-toluene sulfonamide; Figure 2.1) rather than copper sulphate as an oxidizing agent. A measured quantity of filtrate is added with a known quantity of chloramine-T together with potassium iodide solution. After standing for a specified time, the unreacted chloramine-T is measured by titration of liberated iodine against standard sodium thiosulphate solution using starch as an indicator. The difference in amount of chloramine-T in a blank solution and that remaining in the sample solution is equivalent to the lactose in sample. The reactions involved are:

$$CH_3C_6H_4SO_2NClH+H_2O+KI \xrightarrow{(excess)} CH_3C_6H_4SO_2NH_2+ HCl+KIO \text{ (K hypoiodate)}$$

$$KIO+ \text{lactose (-CHO)} \longrightarrow KI+ \text{Lactobionic acid (-COOH)}$$

$$KI+KIO \longrightarrow 2\ KOH+I_2$$

$$I_2+ 2\ Na_2S_2O_3 \longrightarrow 2\ NaI+ Na_2S_2O_6 \qquad \text{(Fox and McSweeny, 1998)}$$

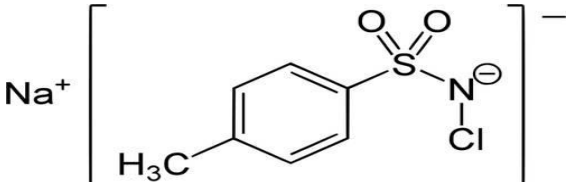


Figure 2.1: Structure of Chloramine-T

(Adopted from en.wikipedia.org/wiki/Chloramine-T)

2.1.2.2 Polarimetric Method

Polarimetric methods are based on the measurement of the specific rotation of lactose in a defatted and deproteinized milk filtrate (AOAC, 1990). The extent of polarization is related to the concentration of the optically active molecules in solution by the equation α = *abc*, where α is the measured angle of rotation, *a* is the optical activity (which is a constant for each type of molecule), *b* is the path length in decimeter and *c* is the concentration (g ml^{-1}). The overall angle of rotation depends on the temperature and wavelength of light used and so these parameters are usually standardized to 20^{o}C and 589.3 nm (the D-line for sodium) (Mathur *et al.*, 1999).

It had been shown that the polarimetric method for determining lactose in commercial condensed whey always gave low results and that was due to the presence of a laevorotatory polypeptide or mixture of polypeptides. This material was formed when acid whey was left in contact with rennet curd. The laevorotatory material could be adsorbed on Fuller's earth and eluted there from by ammonia solution, giving a product with a constant ratio of nitrogen content to optical rotation (House, 1956).

2.1.2.3 Colorimetric Method

The principle involved is that the filtrate containing lactose reacts with certain chemicals to give characteristic colour and from the colour intensity, the corresponding lactose concentration can be calculated using a standard curve made from lactose solution of known concentration. Different chemicals used for colour development and subsequent absorption spectrums are tabulated below (Table 2.3).

Birch and Mwangelwa (1974) reported that two means for determining the lactose and sucrose contents of sweetened condensed milk products were examined. The

first of these was a simple calculation based on polarimetric measurements and gave reproducible results for lactose which agreed well with conventional methods of determination. The second was a novel colorimetric method based on determination of total carbohydrate before and after degradation of lactose with alkali. This gave slightly higher values for lactose in sweetened condensed milk products than conventional methods but could be adopted as a means of analytical control, due to its speed, simplicity and cheapness.

Fox *et. al*. (1962) attempted to estimate lactose by combining Munson- Walker method with colorimetry. Sugars in dairy products were generally determined by either of two basic techniques, Munson-Walker or polarimetric method. The modification of Munson-Walker method reported was based on measuring colorimetrically the amount of cupric ion that was not reduced in the reaction between Fehling's solution and lactose. The protein and fat (when present) of dairy products were removed simultaneously by precipitation with rivanol at neutral pH. After reaction with lactose, the unreacted cupric ion was determined as a cupric-ammonium complex having maximum absorbance at 625 nm. A linear inverse correlation existed between absorbance of the cupric-NH_3 complex from the untreated Fehling's solution and the lactose concentration. The method could be used for products containing sucrose, such as sweetened condensed milk and ice cream.

The semi- micro estimation of lactose alone and in presence of other sugars as described by Malpress and Morrison (1949) appeared to be simple to be adopted in the colorimetry. Reddy and Nath (2011) reported that the colorometric method based on the specific colour reactions of lactose with methylamine was quite suitable for the estimatioin of lactose and in concentrated and lactose hydrolyzed whey.

Table 2.3: Colorimetric method of analysis

Reagents	Measurement	Name of Woker
Anthrone- Sulphuric acid	Colorimetry at 580 nm	Fagen *et al.* (1954)
Phenol- Sulphuric acid	Colorimetry at 480 nm	Popescu *et al.* (1994)
Methylamine- Sodium sulphite	Colorimetry at 540 nm	Malpress and Morrison (1949)
Alkaline potassium ferricyanide	Colorimetry at 420 nm	Popescu *et al.* (1994)
Methylamine–sodium sulphite Glycine–sodium hydroxide	Colorimetry at 540 nm	Nickerson *et al.* (1976)
3,5-Dinitrosalicylic acid	Colorimetry at 570 nm	Popescu *et al.* (1994)

2.1.2.4 Enzymatic methods

The common reaction involved in such methods is the enzymatic hydrolysis of lactose to glucose and galactose, followed by the enzymatic determination of one of the liberated monosaccharides. The difference in the monosaccharide content before and after hydrolysis represents the amount of lactose in the sample. The most common enzymatic method to measure galactose is based on its oxidation by galactose dehydrogenase to galacturonic acid in the presence of nicotinamide-adenine dinucleotide (NAD) that is reduced to NADH, as described by the following reaction:

$$\beta\text{-D-galactose} + NAD^+ \xrightarrow{\beta\text{- D-galactose dehydrogenase}} \text{D-galactono-}\gamma\text{-lactone} + NADH + H^+$$

The absorbance of NADH at 340 nm was calculated as the difference between the readings before and after the addition of the enzyme, galactose dehydrogenase (Coffey and Reithel, 1969 and Lynch and Barbano, 2007).

Shapiro *et al. (*2002) reported that replacement of NAD by thio-NAD and measurement in the visible range at 405 or 415 nm allowed the simultaneous determination of D-galactose concentrations in several samples using microplate-readers, rather than UV spectrophotometers.

Blais and Vailhen (1995) reported that the actions of lactase and glucose oxidase in a buffered solution of iodide, molybdate, and polyvinyl alcohol could be exploited in a microassay format for a chromogenic enzymatic method. Hydrogen peroxide produced was measured by its molybdate-catalyzed reaction with iodide to produce iodine, which formed a colored complex with polyvinyl alcohol.

The most commonly used enzymatic methods are presented in Table 2.4.

2.1.2.5 Biosensors

Lactose biosensors based on immobilized enzymes with electrochemical detection have been reported (Corzo *et al*., 2009). An amperometric lactose biosensor was developed by immobilizing lactase (EC 3.2.1.23) and galactose oxidase (EC 1.1.3.9) in Langmuir–Blodgett (LB) films of poly (3-hexyl thiophene) /stearic acid for estimation of lactose in milk and its products to prevent “lactose intolerance”. The enzyme immobilized LB film was used as working electrode and platinum as reference electrode. The enzyme electrodes had a shelf life more than 120 days. The reusability of electrode was found ten times with 3% loss in current response. The enzyme electrode was characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and kinetic parameters such as pH, temperature and stability. The working electrode may be used for the estimation of lactose/galactose in food and biological fluids (Sharma *et al.*, 2004).

Table 2.4: Enzymatic Methods of Analysis

Analyte	Enzymatic Medium	Detection System	Reported by
Lactose and galactose	Galactosidase Galactose dehydrogenase NAD	Spectrophotometry at 340 nm	Coffey *et al.* (1969) and Lynch *et al.* (2007)
Lactose and galactose	Galactosidase Galactose dehydrogenase *thio*-NAD	Spectrophotometry at 415 nm	Shapiro *et al.* (2002)
Lactose and glucose	Galactosidase, glucoseoxidase Iodide, molybdate, polyvinyl alcohol	Scanning microtitre plate autoreader at 490 nm	Blais and Vailhen (1995)
Lactose	Galactosidase and glucoseoxidase	H_2O_2 Ag–AgCl electrode	Jäger and Bilitewski (2000)
Lactose	Galactosidase, glucoseoxidase, peroxidase, and 5-aminosalycilic acid	Glassy carbon, Pt, and saturated calomel electrodes	Eshkenazi *et al.* (2000)

Some of the biosensor methods are based on the enzymatic hydrolysis of lactose into galactose and glucose, followed by the glucose oxidase-catalyzed conversion of glucose to gluconate and hydrogen peroxide. The measurement of hydrogen peroxide produced may be achieved through different ways, such as by using a Pt electrode (Jäger and Bilitewski, 2000) or oxidation of 5-aminosalycilic acid and further reduction using a glassy carbon electrode (Eshkenazi *et al.,* 2000).

For quantitative determination of lactose in milk a wireless multienzyme biosensor was reported (Yang, 2007). Lactase, glucose oxidase, and catalase were co-immobilized onto a magnetoelastic ribbon-like sensor that was previously coated with a layer of a pH-sensitive polymer. The enzymatic lactose hydrolysis produced glucose that was oxidized to gluconic acid, resulting in the pH responsive polymer-shrinking that caused a shift in the frequency, which was linearly proportional to the lactose concentration.

Furthermore, a lactose biosensor based on cellobiose dehydrogenase from white rot fungi *Trametes villosa* and *Phanerochaete sordida*, which catalyzes the oxidation of lactose and strictly prevents the monosaccharides from the catalytic reaction, has also shown a great potential for the applications in the dairy industry (Stoica, 2006).

2.1.2.6 Chromatography

The different carbohydrates present in dairy products may be determined simultaneously by a considerable number of chromatographic methods, including planar chromatography, gas chromatography (GC) and high-performance liquid chromatography (HPLC).

2.1.2.6.1 Planar Chromatography

It includes both paper and thin-layer chromatography (TLC). These techniques are low-cost, easy to perform, and simultaneously display the overall components present in the sample, in the chromatogram.

2.1.2.6.1.1 Paper Chromatography

Honer and Tuckey (1953) reported a quantitative method for lactose determination in milk by chromatographic analysis. Ethyl acetate, pyridine and water (2.5:1:3.5) were used as solvent system. Direct application of milk to the strip (strips of Whatman No. 1 filter paper , cut into 6 X 22½ in) and subsequent determination of lactose on the chromatostrip after partitioning were desirable features of the procedure. The reproducibility of the method was determined for lactose in pure solution and in milk.

2.1.2.6.1.2 Thin-Layer Chromatography

Lee and Clinton (1976) developed and standardized a semiquantitative chromatographic technique for analysis of foods for lactose content technique using water/chloroform extracts of foods which were spotted onto heat-activated silica gel thin layer chromatographic (TLC) plates, run in butanol/acetic acid/ether/water (18: 12: 3: 2), developed in acidic anisaldehyde, and quantitated.

2.1.2.6.1.3 Gas Chromatography

Olano *et al*. (1986) described a gas chromatographic method using a micropacked column for the analysis of lactose, galactose, lactulose and epilactose in processed milks. The method was evaluated for precision and accuracy using phenyl-β-glucoside as an internal standard. Recoveries near 100% were found for lactulose concentrations higher than 0.1 $mgml^{-1}$, showing coefficients of variation from 5.9 to 9.4%.

2.1.2.6.1.4 High-Performance Liquid Chromatography

Euber and Brunner (1979) reported a method to determine lactose in dairy products by high-performance liquid chromatography. Samples suitable for assay were prepared from the liquid state by deproteinization with trichloroacetic acid (6% wt/vol, final concentration). Lactose was separated by injection of the deproteinized sample onto a *μ*Bondapak/Carbohydrate® column and isocratic elution with an

acetonitrile/water mobile phase. Detection was with a differential refractometer. Sequential injection of a lactose standard and sample, addition of a standard quantity of lactose to the sample, and addition of an internal standard (xylose) to the sample were techniques for quantification.

2.1.2.7 BIS Methods

There are BIS methods for determination of lactose content in milk and milk products.The methods employ Lane-Eynon, Munson- Walker (Gravimatric/ Volumetric) and/ or polarimetric principle (IS: SP: 18; Part XI, 1981).

2.1.3 Starch

2.1.3.1 Starch in ice cream

Unnikrishnan and Narayanan (1987) described a method for starch estimation in ice cream involving precipitation of starch and protein by isopropanol (70%) followed by removal of proteins. Starch was hydrolyzed in presence of acid at 100°C for 2.5 h and estimation of glucose was done using Lane- Eynon's method (BIS 1961). The amount of starch was calculated using the following formula:

$$\% \text{ Starch} = \frac{36.92 * \text{Standard glucose rundown}}{\text{Sample rundown}}$$

DGHS (2005) method adopted from AOAC (1980) involves direct acid hydrolysis wherein the ice cream sample is curdled with alcohol and the precipitated starch is washed with 50% alcohol to remove residual lactose. The precipitated starch is then hydrolyzed to convert into reducing sugars, which is determined by Lane and Eynon's method (BIS 1961). These methods for estimation of starch in ice cream are cumbersome, time consuming and not free from flaws (Padmanabhan and Ramakrishna 1993).

2.1.3.2 Starch in milk and other dairy products

ISO 2000 has recommended a polarimetric method for the estimation of total starch in animal feeds. This method is essentially a modification of the original Evers polarimetric method for the estimation of starch in cereal products (Mitchell 1990). Charles *et al.* (2006) modified this method to certain extent and successfully applied to fluid milk. Later on, this method was validated for its applicability in ice cream, *gulab jamun* mix and *gulab jamun* by Charles *et al.* (2009). Milk products and 40% ethanol soluble fraction were treated with 0.31 M HCl for 20 min under the conditions of boiling and refluxing, respectively. The contents were then filtered. The difference in the optical rotation of these two filtrates is the basis of estimation of starch. While recovery of starch in ice cream and *gulabjamun* mix was nearly quantitative, over estimation has been noted in *gulabjamun*. The method was extensively validated in ice cream in terms of recovery of different types of starches. In general, the recoveries of most starches were more than 90%. Recoveries were very low for modified starches and maltodextrin.

2.1.4 Sucrose

Sugar contents in different dairy products vary depending on the process methodology and the level of sugar added. Since sugar is a major ingredient, its correct determination is necessary. A brief review of work done earlier is reported here.

2.1.4.1 Lane-Eynon Method

This method (IS: SP: 18, PART XI, 1981) principally involves reduction of alkaline cupric salts to red cuprous oxide by reducing sugars on boiling. Sucrose being a non-reducing sugar is first hydrolysed to glucose and fructose in presence of conc. hydrochloric acid and then is titrated against Fehling's solution. From the difference of volume required to titrate before and after inversion, the amount of inverted sugar is calculated. The sucrose content is calculated by multiplying this amount of invert

sugar with a factor 0.95. The process of inversion and titration requires a great level of skill and experience. In 1914, Rakshit replaced hydrochloric acid by citric acid for inversion and used it for determination of sucrose content in presence of lactose in sweetened condensed milk and other milk preparations.

2.1.4.2 Munson- Walker/ Bertrand Method

Laskowski and Jamiolkowska (1970) modified this method for its applicability for estimation of lactose, sucrose and starch in milk baby foods. Two grams of sample was ground in a mortar with 5 ml water at 38-43°C and was quantitatively transferred to a 200 ml volumetric flask using several portions of water at 38-43°C (final vol. up to 90 ml). Five milliliters each of Carrez I and II were added to the solution which was then mixed thoroughly. The volume was made up to mark with distilled water and the contents were allowed to settle and filtered using coarse filter paper. Ten milliliters of filtrate were used for direct determination of lactose by the Bertrand method and 50 ml were hydrolysed for 10 min with 5 ml cone. hydrochloric acid in a 100 ml volumetric flask at 75-80°C, the solution was cooled, neutralized with sodium hydroxide, the volume was brought to 100 ml, and 20 ml of the solution was used for determination of lactose and sucrose. Ten milliliters of concentrated hydrochloric acid was added to the second flask, the solution was heated for 30 min on a boiling water bath with occasional stirring, cooled, neutralized with sodium hydroxide and 10 ml filtrate was used for determination of lactose + sucrose + starch. The amount of flour added was calculated from the starch value on the assumption that wheat flour used for infant formulae contains -76% starches. Accuracy was said to be ± 3% for lactose and sucrose and ± 5% for starch.

2.1.4.3 Polarimetric Method

ISO 2004 describes a polarimetric method for determination of sucrose content in sweetened condensed milk. A test sample is treated with ammonium hydroxide, so as to bring mutarotation of lactose to final equilibrium. It is neutralized and then clarified by successive additions of zinc acetate and potassium hexacyanoferrate(II), followed by filtration. The optical rotation is determined on a portion of the filtrate. On another portion of the filtrate, inversion is induced (based on the Clerget principle) by mild acid hydrolysis of the sucrose, leaving lactose and other sugars virtually unaffected. The optical rotation is determined after inversion.

Reinhardt and Nahrung (1961) developed a rapid method for estimation sucrose content in ice cream using polarimetry. The weighed sample was diluted to 100 ml with alcohol and heated to 70^{o}-80^{o}C for 1 hour in presence of $Ba(OH)_2$ to destroy reducing sugars present. Then the volume was made up with acetic acid and its optical rotation was measured. The sucrose content was calculated by using the formula:

Sucrose (g/kg) = 28.95* L; where, L= optical rotation of sample at 20^{o}C in a 20 cm long polarimeter tube.

2.1.4.4 Two Dimensional TLC

Valentinis and Mattini (1970) developed a method for detection of sucrose, glucose and fructose up to 0.5% level in milk and milk products. The eluants used were n-propanol/ methyl ethyl lactone/ water or methyl ethyl lactone/ acetic acid/water. The eluted fragments from the plate could be estimated by phenol – sulphuric acid method in microgram level.

2.1.4.5 Enzymatic Method

Different enzymes have been employed for estimation of sugars in food products. The results are detected by means of UV spectroscopy or by electrochemical changes produced due to the enzymatic actions. Frank and Christen (1984) developed an enzymatic cryoscopic method to detect sucrose and lactose separately. They incubated the sample with invertase and β- galactosidase for 2 h at 37^{o}C; 50 units of enzyme was required for each sample.

2.1.4.6 Colorimetric Method

The principle is that colour develops in the filtrate containing lactose and inverted sugar with certain chemicals and the intensity of colour can be measured colorimetrically and corresponding concentration of sugars can be calculated. Various works done in this field are listed in Table 2.5.

2.1.4.7 BIS Methods [IS: SP: 18 (Part XI) - 1981]

There are BIS methods for determination of sucrose content in different dairy products like sweetened condensed milk, ice cream, canned *rasogolla and burfi* (IS: SP: 18, PART XI, 1981). The methods employ Lane- Eynon, Munson- Walker and/ or polarimetric principle.

Table 2.5: Colorimetric analysis of sucrose in different dairy products

Sl. No.	Chemical Used for colour development	Conditions	Applicable in Product(s)	Workers
1	Picric acid and Sodium bicarbonate	• Incubation of lactose/ invert sugar solution added with the reagent in boiling water bath for 25 min with for colour development • Intensity of developed colour was measured at 520 nm.	Sweetened Condensed Milk	Perry and Doan (1950)
2	3, 6 dinitropthalic acid	• Zinc carbonate was used to deproteinize the sample	Milk, Dried milk and Condensed milk	Mamose and Mukai (1961)
3	Diphenylamine	• Lead acetate was used to deproteinize the sample • The developed colour was extracted with chloroform and measured for absorbance at 680 nm.	Milk	Garoglio and Stella (1963)
4	Phenol- conc. Sulphuric acid	• Zinc ferrocyanide was used for precipitation. • Incubation was carried out in a boiling water bath for 25 min.	Milk and Milk products	Birch and Mwangelwa (1974)
5	Resorcinol	• Lead acetate was used for precipitation. • Incubation was carried out at 70^{o}C for 40 min. • Developed colour was measured at 490 nm.	Plain Ice craem	Pantulu *et al*. (1976)
6	Resorcinol	• Lead acetate was used for precipitation. • Incubation was carried out at 70^{o}C for 40 min. • Developed colour was measured at 490 nm. • Activated charcoal was used to decolourize the filtrates of coloured products.	Plain ice cream, Coloured ice cream, Sweetened Condensed Milk, *B*urfi	Rao (1994)

2.2 DIABETIC/ DIETETIC FOODS

Diabetic/ dietetic foods were first introduced in the 1960s for people with diabetes as they were advised to follow a diet low in starchy carbohydrates and severely restricted in sugar (Govindji, 2000). In 1984, the Ministry of Agriculture, Fisheries and Foods introduced Food Labelling Regulations which required diabetic foods to have half the amount of rapidly absorbable carbohydrate compared to a standard equivalent. This legislation covered specialist diabetic chocolates, sweets, jams, biscuits and other traditionally sucrose-rich foods. According to Calorie Control Council (2004), an ideal sweetener should have the same sweetness as sucrose. In addition, it should be odourless, colourless, stable and readily soluble in food system. It should be functional, economically feasible, non-carciogenic and non-toxic.

In the 1960s, the diabetic foods introduced were generally of poor quality, with an inferior flavour, and usually contained the bulk sweetener sorbitol. This was used in such high quantities that it could exert an osmotic diarrhoea, even when relatively small amounts of the food were consumed. But afterwards, different artificial sweeteners were declared safe for consumption. There are five artificial sweeteners that have been tested and approved by the U.S. Food and Drug Administration (FDA):

- Acesulfame potassium (also called acesulfame K)
- Aspartame
- Saccharin
- Sucralose
- Neotame

The FSSAI (2012) regulation regarding their use in different sweets is listed in Table 2.6.

In a survey, conducted by Gupta (2010), it was revealed that artificial sweeteners were preferred by 78% of doctors and 92% of nutritionists and they prefer sucralose over aspartame. Top three brands in India are Sugarfree Gold (aspartame), Zero (sucralose) and Sugarfree Natura (sucralose).

Table 2.6: Maximum limit of artificial sweeteners in sweets as per FSSAI (2012)

Sl. No.	Name of the artificial sweetener	Article of food	Maximum limit of artificial sweetener
1	Saccharin Sodium	Sweets (Carbohydrates based and Milk products based) : Halwa, Mysore Pak, Boondi Ladoo, Jalebi, Khoya Burfi, Peda, Gulab Jamun, Rasogolla and similar milk product based sweets sold by any name	500 ppm
2	Aspartame (methyl ester)	Sweets (Carbohydrates based and Milk products based) : Halwa, Mysore Pak, Boondi Ladoo, Jalebi, Khoya Burfi, Peda, Gulab Jamun, Rasogolla and similar milk product based sweets sold by any name	200 ppm
3	Acesulfame Potassium	Sweets (Carbohydrates based and Milk products based) : Halwa, Mysore Pak, Boondi Ladoo, Jalebi, Khoya Burfi, Peda, Gulab Jamun, Rasogolla and similar milk product based sweets sold by any name	500 ppm
4	Sucralose	Sweets (Carbohydrates based and Milk products based) : Halwa, Mysore Pak, Boondi Ladoo, Jalebi, Khoya Burfi, Peda, Gulab Jamun, Rasogolla and similar milk product based sweets sold by any name	750 ppm

Chakraborty (1982) reported about diabetic rasogolla where sucrose was replaced with alcoholic sugar such as sorbitol. Naik and Londhe (2011) have optimized the

levels of artificial sweeteners for preparation of sugar free *kulfi* with 700 ppm of aspartame, 5.87% of sorbitol and 4.24% of maltodextrin to obtain an overall acceptability score of 21.3 with a flavor score of 9.

Several works have been done in NDRI, SRS, Bangalore on development of sweets with artificial sweeteners. Murlidhara (2008) reported that 750 ppm of sucralose, 500 ppm of saccharin or 200 ppm of aspartame with 50, 75 and 75% of sugar replacement, respectively, was the most acceptable level for production of *shrikhand* with non-nutritive sweeteners. Kumar (2008) optimized the technology of dietetic *chhana podo* production. Manufacturing of *basundi* with non-conventional sweeteners had been standardized by Goel (2008). Raval (2008) reported that sucralose (67 ppm) and aspartame (120 ppm) could be used for replacement of sugar in milk chocolate up to 80% level and the remaining sugar could be replaced with sorbitol without affecting the quality.

Based on the above review of literature, it may be seen that though methods are available for sucrose estimation in milk products, their suitability with respect to indigenous milk sweets containing starch as *gulab jamun* and *chhana podo* needs to be evaluated.

Chapter- 3

Materials and Methods

3.0 MATERIALS AND METHODS

The details of materials used and methodologies adopted in the present study are given in this Chapter.

3.1 MATERIALS

3.1.1 SAMPLES

3.1.1.1 *Gulab jamun*

3.1.1.1.1 Preparation of *gulab jamun*

Three hundred grams of *khoa* (prepared from cow milk in the Experimental Dairy of the Institute) was added with 35 g of *maida* (procured from local market). Baking powder (Rex) was added at the rate of 0.8% (on *khoa* basis). The ingredients were then kneaded into dough with water. Small balls were formed and fried in refined sunflower oil (Gemini) at 130^{o}C for 10 min.

Spiking of *gulab jamun* balls with cane sugar / sucrose: The fried *gulab jamun* balls were spiked with cane sugar (procured from Star Bazar, Bangalore) or pure sucrose (AR grade). For this, known amount of *gulab jamun* was thoroughly mixed with sugar/ sucrose solution (made using sugar/sucrose and distilled water in 1:1 ratio) to get 20, 25, 30, 35, 40, 45 and 50% of sugar and 20, 25 and 30% of sucrose in the sample. *Gulab jamun* balls not spiked with sucrose or sugar acted as control.

3.1.1.1.2 Collection of *gulab jamun*

Samples of four different brands of *gulab jamun* were collected from the local market.

3.1.1.2 *Chhana podo*

3.1.1.2.1 Preparation of *chhana podo*

One kg of *chhana* (prepared from cow milk in the Experimental Dairy of the Institute) was added with fine quality *suji* (procured from local market) at the rate of 5% and known amount of sugar (at the rate of 0, 20, 30 and 40%, on *chhana* basis). Then it was blended thoroughly into smooth paste and was filled in aluminum casseroles. Baking was carried out in a baking oven at 155-160^{o} C for about two hour, until the characteristic brown coloured crust was formed. It was then cooled to room temperature.

3.1.1.2.2 Collection of *chhana podo*

Samples of *chhana podo* of three different batches were collected from the milk parlour of the Institute.

3.1.1.3 Dietetic sweets

3.1.1.3.1 Dietetic rabri

Dietetic *rabri* was prepared in the laboratory. Fresh cow milk, standardized to 5% fat and 8.5% SNF was taken in a pre weighed *kadahi* and heated to boiling. Then milk was heated at simmering temperature (80-90ºC) for good skin formation. The skin was removed and was kept at cooler part of *kadahi*. The process was repeated for four to five times until the skin mass came about 10% of the original milk. The skin was collected and taken out of the *kadahi*. Further evaporation of milk was continued until the required concentration (3 to 3.5 times) of milk was reached. At that time the skin was added back to the concentrated milk. Then sugar was added at the rate of 6% of standardized milk. For the preparation of dietetic *rabri*, calculated levels of aqueous solution of artificial sweeteners viz., sucralose/ aspartame/ acesulfame potassium, either to replace sugar completely or partially, were added during the final stages of concentration.

3.1.1.3.2 Dietetic gulab jamun

Samples of dietetic *gulab jamun* were collected from the local market (Star Bazar, Bangalore).

3.1.1.4 Preparation of standard mixed solution

A standard mixed solution was prepared using sucrose, lactose and starch in a ratio of 3:1:2, which approximately is the same as that occurs in gulab jamun and was used for optimization of the method. For dissolving the starch, the protocol used was as same as that for dissolving the macerated gulab jamun sample, i.e. dissolving in distilled water at 80-90^{0}C and filtering the solution through Whatman No. 4 filter paper. The solution was further diluted to get a concentration of 0.09 mg of sucrose/ ml.

3.1.2 Glassware

All the glassware used for the study were cleaned with detergent solution, washed thoroughly under running tap water, rinsed with distilled water and dried completely in hot air oven before using.

3.1.3 Chemicals

All the chemicals used for the experiments were of AR grade and were procured from standard chemical companies. All the reagents needed were prepared fresh before experiment using standard protocol.

3.1.4 Equipements

3.1.4.1 Laboratory Centrifuge

Laboratory centrifuge (R8C, Remi Motors Ltd., Mumbai) was used for estimation of sucrose by Munson-Walker method.

3.1.4.2 Spectrophotometer

Spectrophotometer (Antheli Junior, France) was used for colorimetric estimation of sucrose.

3.1.4.3 Polarimeter

Polarimeter (Bellingham & Stanley, London) was used for measuring the optical rotation of sample filtrate.

3.1.4.4 Kjel plus digestion and distillation assembly

Di SWI-M, KPS-006R, Kjeldahl digestion unit and Kjel plus distillation unit (Pelican Instruments, Chennai) were used to estimate nitrogen contents in samples.

3.1.4.5 Water bath

Water bath (Super fit, India) was used for incubating the sample filtrate for hydrolysis and for estimation of sucrose by colorimetic method.

3.1.4.6 Ovens

Baking oven (Dolar Equipments Pvt. Ltd., Bangalore) was used for baking the *chhana podo*.

Oven (Helios) was used for moisture/TS estimation of the samples.

Hot air oven (Consolidated Electric Industries, Bangalore) was used for drying of glassware.

3.1.4.7 Weighing balance

Weighing balance (Sartorius) was used for weighing the samples for analysis.

3.1.4.8 Grinder/ Mixer

Normal domestic grinder/ mixer (National) was used for grinding the samples and sugar crystals into powder.

3.1.4.9 Vortex Mixer

Vortex mixer (Spinix) was used for thorough mixing of solutions.

3.2 Methods

3.2.1 Sample preparation

3.2.1.1 *Gulab jamun*

Gulab jamun ball was taken out of syrup carefully without damaging the structure. The ball was then placed over filter paper on a funnel for half an hour to drain out the adherent sugar syrup. Then the ball was rolled over a filter paper uniformly for further removal of sugar syrup from the surface. Then it was ground using a glass mortar and pestle. Sample prepared this way was used for further analysis. The pictorial presentation of sample preparation for *gulab jamun* is presented in Figure 3.1.

3.2.1.2 *Chhana podo*

Chhana podo was cut into small pieces which were ground into powder using the grinder/ mixer to obtain an even and uniform composite sample. Such composite sample was used for all the analysis.

3.2.1.3 Dietetic *rabri*

The sample was mixed thoroughly before use.

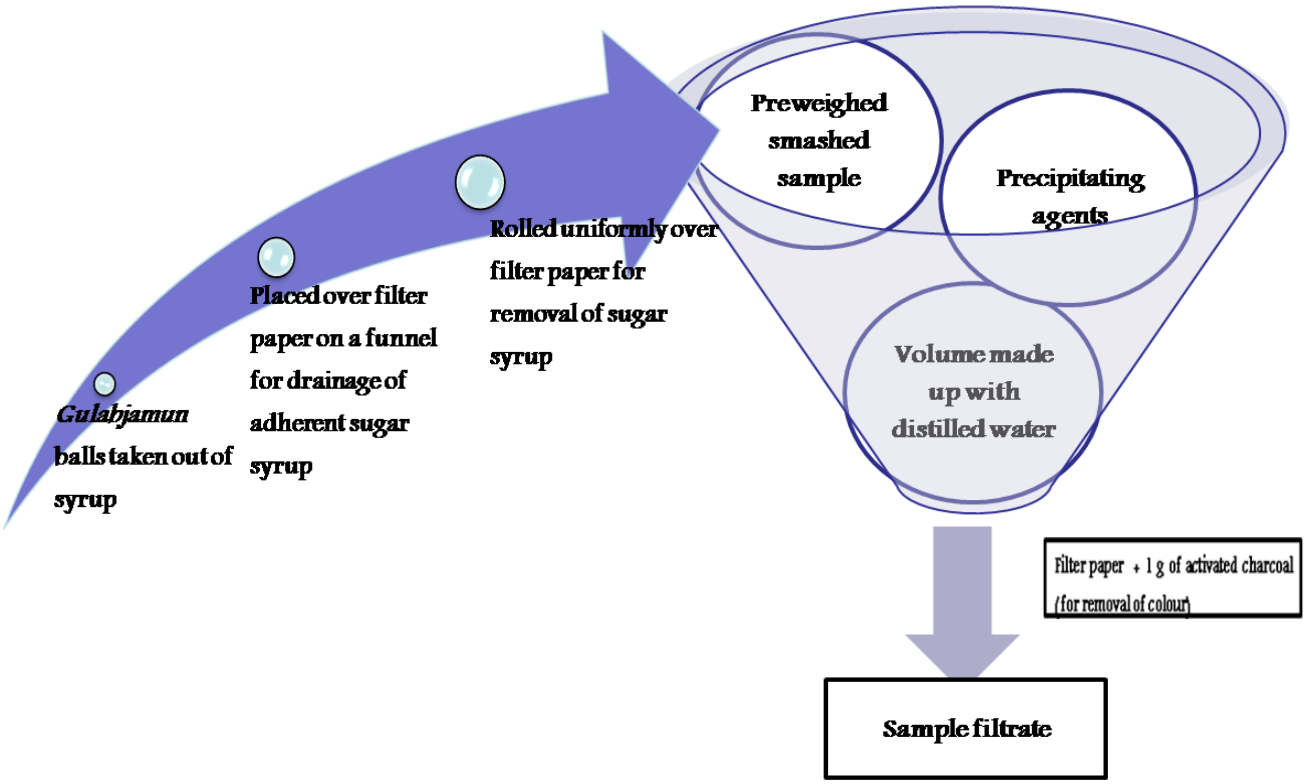


Figure 3.1: Sample preparation for *gulab jamun*

3.2.2 Estimation of Sucrose

The methods adopted with some modification for estimation of sucrose in *gulab jamun* and *chhana podo* are given below.

3.2.2.1 Lane-Eynon Method

The method adopted was as per IS: SP: 18; Part XI (1981) which was given for estimation of sucrose in canned *rasogolla*.

3.2.2.1.1 Reagents

3.2.2.1.1.1 Carrez I (Zinc acetate) solution- Crystalline zinc acetate [$Zn(C_2H_3O_2)_2$, 2 H_2O] (219 g) was added with distilled water and 30 ml of glacial acetic acid and volume was made up to 1000 ml with distilled water.

3.2.2.1.1.2 Carrez II (Potassium ferrocyanide) solution- Potassium ferrocyanide (106 g) was made up to 1000 ml with distilled water.

3.2.2.1.1.3 Dilute ammonia solution- Ten millilitres of concentrated ammonia (sp.gr. 0.88) was diluted to 100 ml with distilled water.

3.2.2.1.1.4 Dilute acetic acid solution- Glacial acetic acid was diluted approximately to equivalent strength of dilute ammonia solution.

3.2.2.1.1.5 Fehling's solution-

3.2.2.1.1.5.1 Fehling's A- Copper sulphate [$CuSO_4$, $5H_2O$] (69.278 g) was dissolved in distilled water with addition of 1 ml of concentrated H_2SO_4 (sp.gr. 1.84) and made up to 1000 ml. The solution was filtered through glass wool.

3.2.2.1.1.5.2 Fehling's B- Rochelle salt [potassium sodium tartarate- $KNaC_4H_4O_6$, $4H_2O$] (346 g) and 100 g of sodium hydroxide were dissolved in distilled water and volume was made up to 1000 ml. The solution was allowed to stand for 2 days followed by filtration though glass wool.

3.2.2.1.1.6 Stock solution of invert sugar – Nine point five gram of sucrose was dissolved in 100 ml distilled water and 5 ml of concentrated hydrochloric acid was added to the solution in a 1000 ml volumetric flask. The solution

was allowed to stand at 20-25^{o}C for 3 days. Then the volume was made up to the mark with distilled water.

3.2.2.1.1.7 Standard solution of invert sugar- Twenty millilitres of stock solution was neutralized with standard sodium hydroxide solution (0.1 N) using litmus paper and then was diluted with distlilled water suitably so that 15-50 ml of the solution was required to titrate 10 ml of Fehling's solution (5 ml each of Fehling's A and B) completely.

3.2.2.1.1.8 Standard sodium hydroxide solution –Standard sodium hydroxide solution (0.1 N) was prepared using analytical grade sodium hydroxide pellets.

3.2.2.1.1.9 Concentrated Hydrochloric Acid- Sp.gr. 1.16

3.2.2.1.1.10 Methylene blue indicator- Methylene blue (0.2 g) was dissolved in distilled water and the volume was made up to 100 ml.

3.2.2.1.2 Method

3.2.2.1.2.1 Standardization of Fehling's solution with standard invert sugar solution- Fehling's solution (5 ml each of Fehling's A and B) was titrated against the standard invert sugar solution under boiling condition in presence of methylene blue indicator. The volume required (in ml) was noted down.

3.2.2.1.2.2 Filtrate preparation- Forty grams of the thoroughly ground sample (*gulab jamun/chhana podo*) taken in a beaker was added with 50 ml of hot (80^{o}-90^{o}C) distilled water. It was then quantitatively transferred to a 250 ml volumetric flask with warm distilled water (60^{o}C) up to a volume of 120-150 ml. It was mixed thoroughly and was cooled to room temperature. Five milliliters of dilute ammonia solution was added to it and was allowed to stand for 15 minutes after proper mixing. Afterwards, the contents were neutralized with dilute acetic acid solution. Carrez I and II (12.5 ml each) were added to the solution and the contents were mixed thoroughly. The volume was made up to mark with distilled water and the contents were allowed to settle and filtered using Whatman No. 04 filter paper (B_1).

Fifty milliliters of the filtrate was taken in a 100 ml volumetric flask and 5 ml of concentrated hydrochloric acid was added to it followed by incubation at 68^{o}C for 5 minutes for inversion. Then it was cooled to room temperature and neutralized with dilute sodium hydroxide using litmus paper. The volume was made up to mark with distilled water (A_1). B_1 and A_1 were approximately diluted so that 15-50 ml was required to neutralize the copper salt present in 10 ml of Fehling's solution and the diluted solutions were marked as B_2 and A_2, respectively.

3.2.2.1.2.3 Titration- Ten milliliters of Fehling's solution (5 ml each of Fehling's A and B) was titrated against B_2 and A_2 under boiling condition in presence of methylene blue indicator. The titre values (in ml) were noted down.

3.2.2.1.2.4 Calculation- The percentage of sucrose was calculated by the following formula:

% Sucrose (by weight) = $20*[W_1/W_2]*[2f_2/V_2-f_1/V_1]$

Where,

W_1= weight in mg of sucrose (from standard invert sugar solution) equivalent to 10 ml of Fehling's solution

W_2= weight in g of material taken for the estimation

f_2= dilution factor for A_2 from A_1

V_2= volume in ml of A_2 required to titrate 10 ml of Fehling's solution

f_1= dilution factor for B_2 from B_1

V_1= volume in ml of B_2 required to titrate 10 ml of Fehling's solution

3.2.2.2 Munson-Walker/ Bertrand Method

The method adopted was as per Weinmann's (1944) modification.

3.2.2.2.1 Reagents

3.2.2.2.1.1 Carrez I(Zinc acetate) solution- as described in 3.2.2.1.1.1

3.2.2.2.1.2 Carrez II (Potassium ferrocyanide) solution- as described in 3.2.2.1.1.2

3.2.2.2.1.3 Ferric ammonium sulphate solution in 20% sulphuric acid- Ferric ammonium sulphate [$Fe(NH_4)(SO_4)_2$] (240.9 g) was dissolved in sufficient quantity of hot distilled water and was added with 200 ml of concentrated H_2SO_4. It was then cooled, filtered and diluted to a final volume of 1000 ml.

3.2.2.2.1.4 Standard 0.1 N potassium permanganate solution- Previously dried potassium permanganate (4.9037 g) was dissolved in distilled water and diluted to 1000 ml. It was then standardized against sodium oxalate and made acidic with sulphuric acid.

3.2.2.2.1.5 Fehling's solution- as described in 3.2.2.1.1.5

3.2.2.2.1.6 Standard Sodium Hydroxide solution - as described in 3.2.2.1.1.8

3.2.2.2.1.7 Concentrated Hydrochloric Acid- as described in 3.2.2.1.1.9

3.2.2.2.2 Method

3.2.2.2.2.1 Filtrate preparation- Two grams of properly ground sample (*gulab jamun/chhana podo*) was weighed accurately in a beaker and was dissolved in 5 ml of distilled water (38^{o}-43^{o}C). It was then quantitatively transferred to a 200 ml volumetric flask with distilled water (38^{o}-43^{o}C) up to a final volume of 90 ml. It was mixed thoroughly and was cooled to room temperature. Five milliliters each of Carrez I and II were added to the solution which was then mixed thoroughly. The volume was made up to mark with distilled water and the contents were allowed to settle and filtered using Whatman No. 04 filter paper.

Fifty milliliters of the filtrate was taken in a 100 ml volumetric flask and 5 ml of concentrated hydrochloric acid was added to it followed by incubation in a water bath at 75^{o}-80^{o}C for 10 minutes for inversion. It was then cooled to room temperature and neutralized with standard sodium hydroxide solution (0.1 N) using litmus paper. The volume was made up to mark with distilled water.

3.2.2.2.2.2 Precipitation and Titration- Five milliliter aliquots of sample filtrate were taken in 15 ml centrifugation tubes. Five milliliters of mixed Fehling's solution was added to it and the tubes were placed in a boiling water bath exactly for 3 minutes followed by cooling under running water for another 3 minutes. Then the tubes were centrifuged at 2000-3000 rpm for 3 minutes and the supernatant was decanted off. Twelve milliliters of distilled water was added and again centrifuged for 3 minutes. This process was repeated for two times. After final decantation, the precipitate was dissolved in ferric ammonium sulphate solution and was titrated against standard 0.1 N potassium permanganate solution. Blank testing was carried out by replacing the sample aliquot by 5 ml of distilled water. The blank titre values were subtracted from the sample titration values.

3.2.2.2.2.3 Calculation-

1 ml of 0.1 N $KMnO_4$= 0.00635 g of Cu

Hence, the weight of Cu in precipitate was V*0.00635 g, where V is the titre value.

From the quantity of reduced copper, the amount of glucose was found by reference to copper-glucose equivalent table and from this glucose value, amount of lactose was obtained by multiplying with a factor, 1.786.

Now from difference, the reducing power of the solution was measured in terms of lactose and sucrose percentage was calculated as per the following formula:

% Sucrose= W_1/W_2*0.95

Where,

W_1= weight of lactose in precipitate (in mg)

W_2= weight of sample taken for analysis (in g)

3.2.2.3 Polarimetric Method

The method adopted was as per IS: SP: 18; Part XI (1981) for estimation of sucrose in canned *rasogolla*.

3.2.2.3.1 Reagents

3.2.2.3.2 Mercuric nitrate solution - 10%.

Note- The solution tends to become acidic with progressive time due to deposition of mercury salts. So dilute alkali was added occasionally to get slight permanent precipitate and the solution was re-filtered before use using Whatman No. 01 filter paper.

3.2.2.3.3 Hydrochloric Acid - Sp.gr. 1.102 at 20^{o}C.

3.2.2.3.4 Standard sodium hydroxide solution – Standard sodium hydroxide (0.5 N) solution was prepared by using analytical grade sodium hydroxide pellets.

3.2.2.3.2 Method

Twenty grams of thoroughly ground sample (*gulab jamun/chhana podo*) taken in a beaker was quantitatively transferred to a 100 ml volumetric flask with distilled water (38^{o}-43^{o}C). The volume was made up to mark with distilled water and the contents were allowed to settle and filtered through Whatman No. 04 filter paper. First 5 ml of filtrate was discarded.

Fifty milliliters of the filtrate taken in a 100 ml volumetric flask was added with 25 ml of distilled water and 5 ml of 10% mercuric nitrate solution. The contents were mixed thoroughly without delay and neutralized with standard sodium hydroxide solution (0.5 N) using litmus paper. Care was taken to

avoid alkaline reaction. Finally, the volume was made up to the mark with distilled water and the solution was filtered thoroughly using Whatman No. 04 filter paper.

Twenty five milliliters of the filtrate was taken in a 50 ml volumetric flask and was diluted to the mark with distilled water. Then it was polarized at 20^{o}C in 200 mm tube (D).

Fifty milliliters of the filtrate was taken into a 100 ml volumetric flask with 10 ml of concentrated hydrochloric acid and was kept at temperature more than 20^{o}C for 24 hours. The volume was made up to mark with distilled water and was polarized at 20^{o}C (I).

Calculation:

The percentage of sucrose was calculated by the following formula:

% Sucrose= [{D-(5/4)*I}/Q]*[(V-v)/ (L*M)]

Where,

D= Direct polarimeter reading before inversion

I= Direct polarimeter reading after inversion

Q= Inversion Division factor [= 0.8825 at 20^{o}C and 589.3 nm]

V= Volume in ml to which the sample is diluted before filtration

v = Volume of precipitate [=M/100*(1.08*%Fat+1.55*% Protein]

L= Length of polarimeter tube in decimeter

M= Weight of sample taken for estimation (in g)

3.2.2.4 Seliwanoff's colorimetric method

The colorimetric method of Pantulu *et al*. (1981) reported for ice cream was followed.

3.2.2.4.1 Reagents

3.2.2.4.1.1 Lead acetate solution- 40% solution (aqueous)

3.2.2.4.1.2 Resorcinol solution- 0.1% solution (aqueous)

3.2.2.4.1.3 Concentrated hydrochloric acid- 12 N

3.2.2.4.1.3.1 Standard sucrose solution- Twenty miligrams of pure sucrose was dissolved in distilled water and was diluted to a final volume of 100 ml to get a concentration of 0.2 mg/ml.

3.2.2.4.2 Method

3.2.2.4.2.1 Filtrate preparation- Two grams of properly mixed, macerated sample (*gulab jamun/ chhana podo/ rabri*) was taken in a 500 ml volumetric flask and the volume was made up to the mark with distilled water after addition of 1 ml of 40% lead acetate solution. The solution was filtered through Whatman No. 04 filter paper. One milliliter of filtrate was further diluted to 10 ml with distlled water in 10 ml volumetric flask. The contents were vortexed for complete and thorough mixing. In case of samples containing added colour, the filtrate was passed through 1 g of activated charcoal to get a colourless clean filtrate.

3.2.2.4.2.2 Colorimetric estimation of sucrose- Aliquots (0.5 and 1.0 ml) of sample filtrate were taken in test tubes. Volume was made up to one milliliter with distilled water. Two milliliters of 0.1% aqueous solution of resorcinol was added followed by the addition of six milliliters of concentrated (12N) hydrochloric acid. The contents were vortexed and incubated at 70^0C for 30 min period for optimal colour development. The solution was cooled to room temperature and the developed colour was measured for absorbance at 490 nm using a spectrophotometer. The

level of sucrose was quantified with the help of a standard curve drawn using standard sucrose solution.

3.2.2.4.2.3 Preparation of standard curve- A standard curve was prepared using standard sucrose solution of known concentration (0.2 mg/ml). Different aliquots, ranging from 0.1 to 1 ml, were used for colour development. The procedure of the colour development and measurement were same as detailed above; incubation at 70^0C was done for 30 min. The levels of sucrose in mg of the aliquots were placed along the X-axis and the observed optical densities were placed along the Y-axis. The graph was drawn using Microsoft Office Excel 2007. The R^2 value obtained was 0.994. The standard curve was validated using different aliquots of sucrose solution of known concentration.

The colorimetric method of estimation has been presented as Flow chart in Figure 3.2.

3.2.2.4.3 Calculation-

The percentage of sucrose was calculated by the following formula:

% Sucrose= (V-v)*W/V/M

Where,

V= Volume in ml to which the sample is diluted before filtration

v = Volume of precipitate [=M/100*(1.08*%Fat+1.55*% Protein]

W= weight of sucrose in aliquot quantified from standard curve (in mg)

V= Volume of aliquot taken (in ml)

M= Weight of sample taken for estimation (in g)

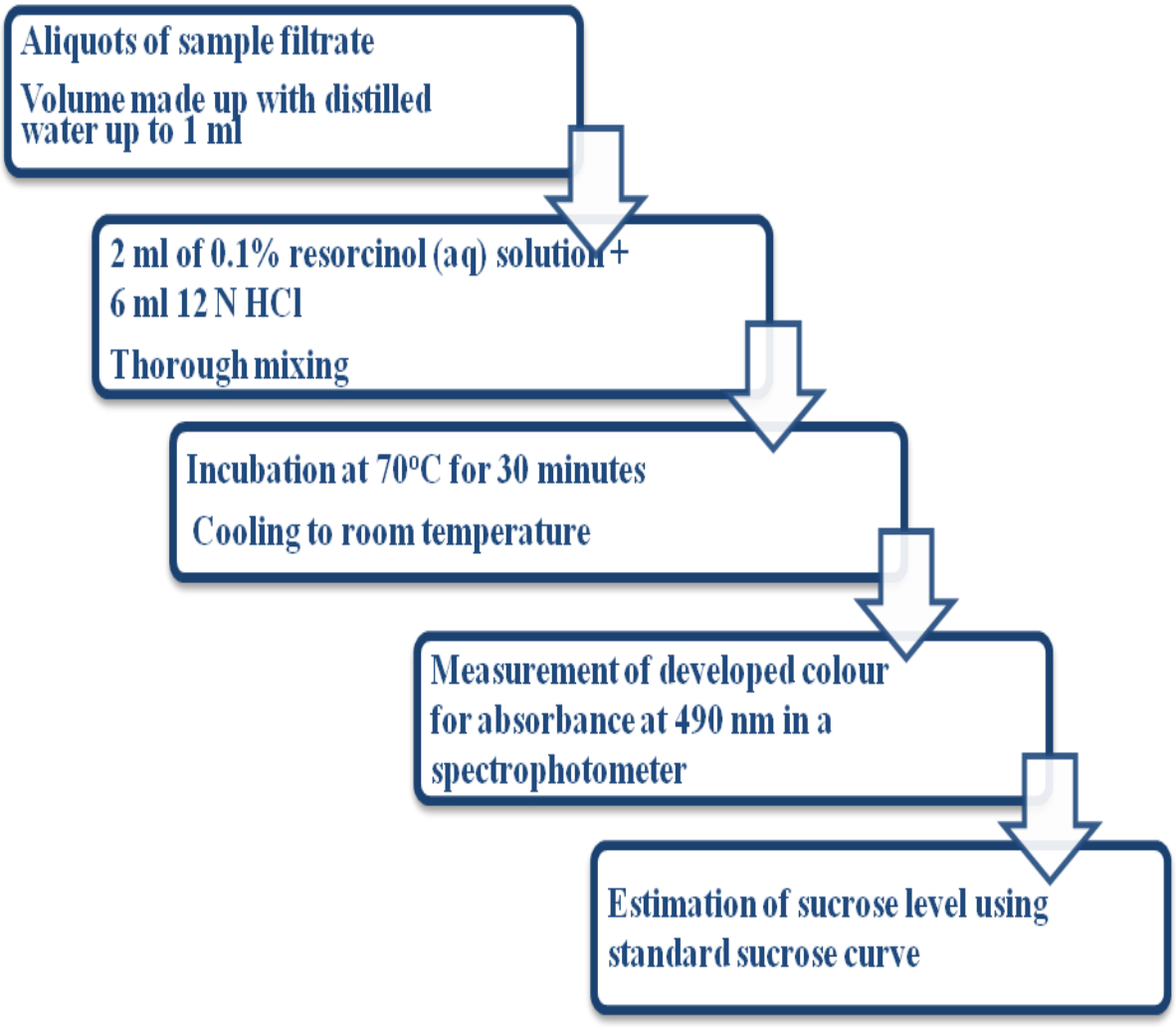


Figure 3.2 : Seliwanoff's colorimetric method for estimation of sugar

3.2.3 Physico chemical analysis of samples

3.2.3.1 Moisture estimation (IS: SP: 18; Part XI, 1981)

One gram of thoroughly mixed, macerated sample (*gulab jamun/ chhana podo/ rabri*) was taken in a pre-weighed dry aluminum dish. It was then dried in a hot air oven at 102^0C for 5 hours followed by cooling in a desiccator and weighed. Heating at 100^0C $\pm 2^0$C for 30 min, cooling and weighing was repeated until the loss in weight was not more than 0.5 mg. From the difference in initial weight and final weight, the moisture content was calculated.

$$\text{Moisture (\%)} = \frac{W_2 - W_1}{W_1} * 100$$

Where, W_1= Weight of sample taken for analysis

W_2= Weight of sample after drying

3.2.3.2 Fat estimation (IS: SP:18; Part XI, 1981)

Two grams of sample (*gulab jamun/ chhana podo/ rabri*) was taken in a beaker and was added with 10 ml of concentrated hydrochloric acid. The contents were mixed well and placed over a steam bath for digestion. After that, the material in beaker was quantitatively transferred to a Mojonnier flask with 15 ml ethyl alcohol and 25 ml each of diethyl ether and petroleum ether. After thorough shaking, the contents in tube were allowed to stand for 30-40 min until clear separation of layers observed. The solvent layer was decanted in a pre-weighed beaker, containing pumice stones. The extraction of the aqueous layer was repeated twice using 15 ml each of the ethers. The solvent in the beaker was dried on steam bath and the residual fat was further dried in the oven at 100^0C $\pm 2^0$C for one hour followed by cooling in desiccators and then weighed. Fat percentage was calculated using the following formula:

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample taken for analysis}} * 100$$

3.2.3.3 Protein estimation (IS: SP:18; Part XI, 1981)

Approximately 0.5 g of sample (*gulab jamun/ chhana podo/ rabri*) was weighed accurately and transferred to the Kjeldahl tube. Five gram of digestion mixture (potassium sulphate and copper sulphate- 20:1) and 12 ml of concentrated sulphuric acid were added to the flask. The contents were digested in Kjedahl digestion unit until clear solution was obtained. About 30 ml of distilled water was added to the tube along the sidewalls. The tube was placed in Kjeldahl distillation unit. Auto measured quantity of 50% (w/v) standard sodium hydroxide solution was added to it to make the solution alkaline. The contents were steam distilled and liberated ammonia was collected in 25 ml of saturated boric acid solution containing 2-3 drops of mixed indicator (methyl red and methylene blue). After completion of distillation, the distillate was titrated against 0.1 N standard sulphuric acid to an end point of purple colour. A blank test was carried simultaneously using all the reagents and pure analytical grade sucrose in place of the test material. The protein percentage was calculated as per below:

$$\text{Protein (\%)} = \frac{(A-B)*1.4*6.25*N}{W}$$

Where,

A = Reading for sample (ml)

B = Reading for blank (ml)

N= Normality of H_2SO_4

W = Weight of sample taken for analysis (g)

3.2.3.4 Lactose estimation

Lactose was estimated in *khoa* and *chhana* as per the Lane- Eynon method (IS:SP: Part XI,1981) and the level in *gulab jamun* and *chhana podo* was calculated by ingredient balance. The level in the products was also determined by Lane- Eynon and Munson-Walker-Bertrand's method.

3.2.3.5 Correction for volume of precipitate

The volume for precipitate in the filtrate obtained in Lane- Eynon, Munson-Walker, polarimetric and colorimetric methods was corrected assuming that 1 g of protein occupies 0.8 ml and 1 g of fat occupies 1.075 ml of space.

3.2.4 Statistical analysis

Data obtained during the present study were subjected to two way ANOVA and standard error calculations as described by Snedecor and Cochran (1994) and employing MS- EXCEL computer package.

Chapter- 4

Results and Discussion

4.0 RESULTS AND DISCUSSION

A number of Indian milk sweets such as *gulab jamun*, *burfi, peda, kheer, payasam* and *halwa*s are made up of milk solids, sugar and starch in the form of *maida* or *suji*. Some of these sweets also contain ingredients such as nuts, raisins and added colours. There is a lot of variation in the types and levels of ingredients used and methods of preparation which result in a wide variation in the physico-chemical and sensory quality of these products. In this regard, variation in sugar level appears to be the highest.

Exact estimation of sugar content is necessary for checking complete compliance to standards and to meet labeling pre-requisites. As a common practice, total carbohydrate in a food product is estimated by difference method. Sugar level is calculated from the ingredients balance, i.e., level of sugar added and the mass of the final product.

In the conventional methods (Lane-Eynon, Munson- Walker or polarimetric method) for determination of sucrose in milk and milk products, inversion of sucrose is done and then the difference in either reducing property or optical activity is measured as compared to lactose. However, they have not been tested for their efficiency for sugar estimation in products containing lactose and starch. In India, most of the milk sweets are either *khoa* based or *chhana* based. In this project, two sweets, viz. *gulab jamun* and *chhana podo,* which are based on *khoa* and *chhana*, respectively were chosen for evaluation of different methods for sugar estimation. These products also contain starch in addition to added sugar and milk sugar, lactose. *Gulab jamun* samples were prepared in the laboratory under controlled conditions. Samples of a few brands of *gulab jamun* were also procured from the market. *Chhana podo* samples were collected from the milk parlour in the Institute and were also prepared in the Experimental Dairy plant using *chhana* made from cow milk. Samples of *gulab jamun* and *chhana podo* (without any sugar) served as control and were tested with all the methods adopted to check their specificity for sucrose.

4.1 Evaluation of methods

4.1.1 Selection of methods

The methods adopted for evaluation in the present study were:

4.1.1.1 Lane-Eynon Method: This method principally involves quantitative reduction of alkaline cupric salts present in Fehling's solution (copper sulphate in sodium potassium tartrate) to red cuprous oxide by titration with lactose, a reducing sugar, on boiling. Methylene blue is used as the redox indicator. From the amount of copper salt reduced, the quantity of lactose is calculated. The sucrose content is calculated by taking the difference in the reducing sugar before and after inversion and multiplying it with a factor 0.95 (Mathur *et al.*, 1999).

$$\text{Reducing sugar} + Cu^{2+} + \text{Base} \xrightarrow{\blacktriangle} \text{Oxidized sugar} + Cu_2O \text{ (precipitate)}$$

4.1.1.2 Munson Walker Method: Carbohydrates are oxidized in the presence of heat and an excess of copper sulphate and alkaline tartrate under carefully controlled conditions which leads to the formation of a copper oxide precipitate. The amount of precipitate formed is directly related to the concentration of reducing sugars in the initial sample (Srivastava, 2010). The wet precipitate of cuprous oxide is dissolved in known quantity of saturated solution of ferric ammonium sulphate. This solution is then titrated with the standard solution of potassium permanganate. As one milliliter of 0.1 N potassium permanganate is equivalent to 0.00635 g of copper, the weight of cuprous oxide in mg present in the known quantity of filtrate used can be determined from the titre value (Mathur *et al.*, 1999).

4.1.1.3 Polarimetric Method: Polarimetric methods are based on the measurement of the specific rotation of lactose in a defatted and deproteinized milk filtrate (AOAC, 1990). The extent of polarization is related to the concentration of the optically active molecules in solution by the equation $\alpha = abc$, where α is the measured angle of rotation, a is the optical activity (which is a constant for each type of molecule), b is the path length in decimeter and c is the concentration (g ml^{-1}). The overall angle of rotation depends on the temperature and wavelength of light used and so these

parameters are usually standardized to 20^{o}C and 589.3 nm (the D-line for sodium) (Mathur *et al.*, 1999).

4.1.1.4 Seliwanoff's Colorimetric Method: The principle of this method is that colour develops in the filtrate containing ketonic sugar on reacting with resorcinol-hydrochloric acid reagent and the intensity of colour can be measured colorimetrically and the concentration of sugar can be calculated (Pantulu, 1981).

4.1.2 Evaluation with *gulab jamun* samples

For evaluating the above four methods, *gulab jamun* samples spiked with known amount of cane sugar were taken for analysis. For this, known amount of *gulab jamun* was thoroughly mixed with sugar solution (made using sugar and distilled water in 1:1 ratio) to get 30, 40 and 50% of sugar in the sample. *Gulab jamun* balls not spiked with sugar acted as control. The samples were tested for sucrose levels by adopting the methods of Lane-Eynon method (IS: SP: 18; Part XI- 1981); Munson-Walker-Bertrand's method: modified by Weinmann (1944); Polarimetry (IS: SP: 18; Part XI- 1981) and Seliwanoff's colorimetric method (Pantulu *et al.*, 1981). Sucrose levels estimated by the different methods in the control sample of *gulab jamun* are presented in Table 4.1. It may be seen from the Table that control samples of *gulab jamun* (without any sugar) showed the presence of sugar when tested by Lane- Eynon and Munson- Walker methods while the level showed by the polarimetric method was on the negative side. However, the samples did not show the presence of sugar when tested by colorimetric method.

The data obtained for spiked samples are shown in Table 4.2. The differences in sugar content between the calculated and estimated levels, as estimated by the colorimetric, Lane- Eynon and Munson- Walker methods were positive, while it was negative in the polarimetric method. On testing with two way ANOVA, it has been observed that the difference between observed value and actual level was not significant for colorimetric method and Munson-Walker method ($P > 0.05$). However, the difference was significant for the other two methods i.e., Lane- Eynon method and polarimetric method ($p < 0.05$). When the standard error (SE) of data was taken

into consideration (Table 4.3), Seliwanoff's colorimetric method with a lower SE appeared to give better accuracy.

Table 4.1: Evaluation of specificity of methods for sucrose in control samples of *Gulab jamun* (without added sugar)

Methods	Observed sucrose level (%)	
	WB	DB
Lane- Eynon method	8.70 ± 1.48	14.68 ± 2.69
Munson-Walker method	5.29 ± 1.84	7.10 ± 2.78
Polarimetry	(-) 2.99 ± 0.70	(-) 3.26 ± 0.86
Colorimetry	0	0

Number of trials: 3

Table 4.2: Estimation of sucrose in *gulab jamun* by different methods

Methods	Statistical Mean (%)		
	Calculated level	Estimated level	Difference
Lane- Eynon method	40.18^{a}	49.92^{b}	9.74
Munson-Walker method	40.18^{a}	41.36^{a}	1.19
Polarimetry	38.85^{b}	7.77^{a}	(-) 31.08
Colorimetry	39.99^{a}	46.04^{a}	6.05
$CD_{0.05}$ =8.74			

Values with different superscripts vary significantly ($P < 0.05$)

Table 4.3: Standard Error for estimation of sucrose levels in *gulab jamun* by the Colorimetric and Munson- Walker methods

Method	Standard Error
Colorimetric	8.47
Munson-Walker	11.52

4.1.3 Evaluation with *chhana podo* samples

On a similar fashion, four different batches of *chhana podo,* prepared in the Experimental Dairy plant with varied sugar levels (0, 20, 30 and 40% on *chhana* basis) were tested for evaluation of the methods of sucrose estimation. *Chhana podo* prepared without addition of sucrose served as control and this sample did not show any sucrose level in it when estimated by colorimetric method; it was overestimated in the case of Lane- Eynon and Munson- Walker methods and was negative when tested by the polarimetric method. Sucrose levels estimated by the different methods in the control sample of *chhana podo* (without sugar) are presented in Table 4.4.

The data obtained for spiked samples are shown in Table 4.5. On testing with two way ANOVA, the same trend was observed as that of *gulab jamun*, i.e., no significant variation for colorimetric and Munson- Walker methods ($P > 0.05$) unlike the other two methods. Here also Seliwanoff's colorimetric method appeared to be more accurate with a lower SE (Table 4.6).

Table 4.4: Evaluation of specificity of methods for sucrose in control sample of *Chhana podo* (without added sugar)

Method	Observed sucrose level (%)	
	WB	DB
Lane- Eynon	5.33 ± 1.02	9.63 ± 0.89
Munson-Walker	3.87 ± 1.19	5.88 ± 2.12
Polarimetric	(-) 1.06 ± 0.82	(-) 1.98 ± 0.66
Colorimetric	0	0

Number of trials: 3

Table 4.5: Estimation of sucrose in *chhana podo* by different methods

Treatments	Statistical Mean (%)	Difference
Calculated Level	26.67^{ab}	___
Lane- Eynon method	31.26^{c}	4.59
Munson-Walker method	28.43^{b}	1.76
Polarimetry	14.21^{d}	-12.46
Colorimetry	30.18^{a}	3.52
$CD_{0.05}$ =4.56		

Values with different superscripts vary significantly (P < 0.05)

Table 4.6: Standard Error for estimation of sucrose levels in *chhana podo* by the Colorimetric and Munson- Walker methods

Method	Standard Error
Colorimetric	5.5
Munson-Walker	7.58

4.1.4 Interpretation of the results

The results of the trials have shown that theconventional methods (Lane- Eynon, Munson- Walker, polarimetric) were not of help for estimation of sucrose in milk sweets like *gulab jamun* and *chhana podo* probably due to the interference from the hydrolysis products of the starch formed during inversion. All the methods involved sample preparation, i.e., filtrate containing the soluble carbohydrates. In this regard, different precipitation techniques were used for precipitation and the final starch level in the filtrate depends on the method of precipitation. The methods of Lane-Eynon, Munson-Walker and polarimetry also involved inversion of sugars where degree of inversion of sugars in the presence of starch may not be consistent. Seliwanoff's

method appears to help in this regard, as there is no step of inversion and more importantly, a specific reaction occurs between the keto group of sucrose and the resorcinol-HCl reagent for colour development.

4.1.4.1 Lane-Eynon method

This method principally involves the quantitative reduction of alkaline cupric salt (copper sulfate in sodium potassium tartrate) to red cuprous oxide by titration with reducing sugar, on boiling (Fig. 4.1). When this method was employed for estimation of sugar in *gulab jamun* and *chhana podo* samples with known amount of sugar, an overestimation was observed. Even the control samples (without any sugar) showed the presence of sugars. The control samples (without sugar) of *gulab jamun* and *chhana podo* showed an over estimation of 8.77 and 5.33%, respectively. The estimated sugar level in case of *gulab jamun* spiked with 40% sugar was 50% (difference + 10%) and in case of *chhana podo* with 27% sugar, the level estimated was 31% (difference + 4.6%). Such an overestimation could be due to formation of reducing sugars from starch during inversion. Thus, though these methods worked well for estimation of sugar in plain ice cream and sweetened condensed milk (Pantulu *et al,* 1981) and in coloured ice cream and *burfi* (Rao, 1994), the products without starch, it did not work well in the case of *gulab jamun* and *chhana podo* due to the presence of starch.

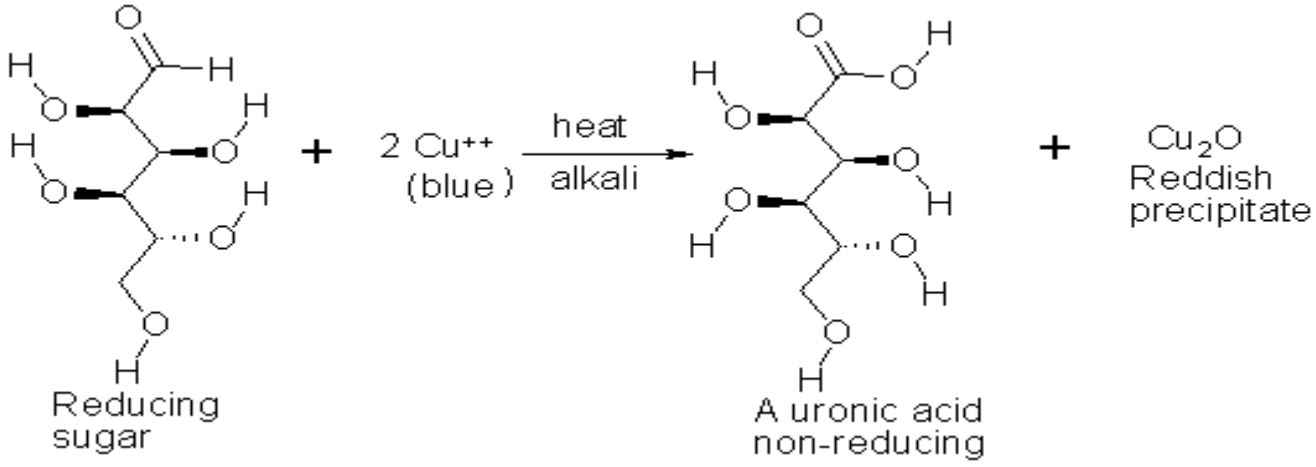


Figure 4.1: Reaction of Reducing Sugars with Fehling's solution
(adopted from: www.cfs.purdue.edu)

4.1.4.2 Munson- Walker method

This method employs the same principle as of Lane-Eynon method up to the stage of Fehling's reaction. Later the cuprous oxide precipitated was separated by centrifugation, dissolved in acidic ferric ammonium sulphate solution and titrated against standard potassium permanganate solution (0.1 N) (Srivastava, 2010). From the titre value, weight of equivalent copper is estimated and calculated for glucose. The glucose equivalent is converted to corresponding lactose value and from the differences in the lactose values obtained before and after inversion the result is expressed as sucrose.

Laskowski and Jamiolkowska (1970) applied this method for estimation of lactose, sucrose and starch in milk baby foods and obtained an accuracy of ± 3% for lactose and sucrose and ± 5% for starch. However, such accuracy was not achieved for *gulab jamun* and *chhana podo* in the present study. The method has more steps than that of Lane-Eynon method. As experienced with Lane-Eynon method, it is expected for an over estimation of sucrose by this method also. However, the results obtained in this method agree to a better degree with the calculated levels than with the Lane-Eynon method. It is not understood whether some loss of the precipitate of cuprous oxide into the supernatant or some other factor is responsible for such a trend in the result obtained by this method. In any case, this method appears to be cumbersome and there is a possible loss of the sugar being estimated in the supernatant.

4.1.4.3 Estimation of lactose by Lane- Eynon and Munson-Walker methods

Table 4.7 shows the data on lactose levels of *gulab jamun* and *chhana podo* samples as estimated by Lane- Eynon and Munson-Walker methods. It may be seen from the data that the lactose levels obtained by both the methods were almost the same and agree with the calculated level to a great extent. However, in case of the Munson- walker method, the lower level of overestimation of sucrose as as occurred

in the estimation by Lane- Eynon method could be due to loss of some precipitate or some other unexplainable reasons.

Table 4.7: Estimation of lactose levels in *gulab jamun* and *chhana podo* by Lane- Eynon and Munson-Walker methods

Products	Calculated levels (%)		Observed levels (%)			
			Lane- Eynon method		Munson- Walker method	
	WB	DB	WB	DB	WB	DB
Gulab jamun	6.99 ± 0.21	9.01 ± 0.42	6.91± 0.39	9.07 ± 0.62	6.57 ± 0.63	8.62 ± 0.46
Chhana podo	2.20 ± 0.57	3.25 ± 0.53	2.10 ± 0.42	3.13 ± 0.86	1.99 ± 0.40	3.01 ± 0.17

Number of trials: 4

4.1.4.4 Polarimetric method

The polarimetric method uses the property of reducing sugars to rotate the plane of polarized light owing to the presence of chiral carbon atom. The data on sucrose levels as estimated by this method using control and spiked samples of *gulab jamun* and *chhana podo* are presented in Table 4.1, 4.2, 4.4 and 4.5, respectively. The results show that in control samples of *gulab jamun* and *chhana podo* (without sugar), the optical rotation after inversion was observed to be lower as compared to that obtained before inversion. In case of spiked sample also, as may be seen form Table 4.2 and 4.5, that this method underestimated sugar levels significantly. During the analysis, it was observed that the solution turns turbid after inversion. While this may not interfere in the titrimetric methods, it is likely to affect the polarization of light. The lower degree of optical rotation in the solution after inversion observed in this study could be due to such an effect. Charles *et al* (2009) estimated the total starch content in ice cream, *gulab jamun* mix and *gulab jamun* with a modified ISO method (ISO 6493:2000E), recommended for the estimation of total starch in animal feeds, which includes a step for isolation of starch. However, the polarimetric method evaluated in this study (IS: SP: 18; Part XI- 1981) basically intended for

sucrose estimation in canned *rasogolla* and *burfi* and adopted in the present study did not have a step for removal of starch.

4.1.4.5 Colorimetric method

Seliwanoff's colorimetric method involves the reaction between the ketonic group of sucrose present in the filtrate and HCl- Resorcinol reagent. The ketonic group of sucrose under acidic condition forms a pink colour complex with resorcinol. The specificity of the method for sucrose could be seen from the data given in Table 4.1 and 4.4 for control samples (without sugar) of *gulab jamun* and *chhana podo*, respectively. These samples did not show the presence of sugar when tested by the colorimetric method. However, the method when tested with spiked samples of *gulab jamun* and *chhana podo*, overestimated the level to an extent of 6 and 3.5% respectively.

4.2 Optimization of colorimetric method

From the above results, it may be seen that among the four methods evaluated for sucrose estimation in *gulab jamun* and *chhana podo* with known level of sugar, the colorimetric and Munson- Walker methods estimated the sugar content close to the calculated level. However, considering the SE of data (Table 4.3 and 4.6), the colorimetric method with a lower SE appeared to be better. The results obtained by the colorimetric method were consistent and repeatable. Further, Munson- Walker method is not specific to sucrose, as the control samples (without sugar) of *gulab jamun* and *chhana podo* were shown to have sugar. In this regard, Seliwanoff's method was specific and hence gave consistent results in the experimental samples of *gulab jamun* and *chhana podo*. Hence, efforts were made to optimize this method and the results are presented here.

4.2.1 Standard mixed solution

A standard mixed solution containing sucrose, lactose and starch in the ratio of 3:1:2, which approximately is the same that occurs *gulab jamun* was used for optimization of the method. Sucrose level in the aliquots taken for colour

development was kept in the range from 0.018 to 0.090 mg as this range was observed to show a linear relation between the concentration of sucrose and the optical density. Small variation in the concentration and/ or amount of resorcinol solution did not affect the result. However, the level of the reagent was kept as 2 ml of 0.1% resorcinol solution (aqueous). Earlier studies by Pantulu *et al* (1981) showed that incubation at 70ºC was optimal for colour development. At higher temperature and in the presence of strong acids, aldohexoses may produce hydroxyl methyl furfural. This in turn reacts with resorcinol to give coloured compounds, which may interfere with the estimation. Hence, in the present study, incubation of the samples for colour development was carried out at 70ºC. In addition to temperature, incubation time may also interfere with colour development. Pantulu *et al* (1981) incubated the samples of ice cream and sweetened condensed milk for 40 min. For determination of sugar in coloured ice cream and *burfi*, Rao (1994) incubated the solution for 40 min for colour development. In the present study, incubation was carried out for different periods ranging from 10 to 40 min with increments of 5 min. The initial trials in this regard showed that incubation up to 20 min was not adequate for colour development and incubation for 40 min gave values much higher than the calculated levels. Hence, incubation for 25 to 35 min was carried out in further trials and the results are shown in Table 4.8. The variation with the calculated level was significant for the results obtained with incubation period of 35 min. It may also be seen from the data that among the various duration used for colour development, an incubation time of 30 min was observed to give levels of sucrose within ± 1% from the calculated levels present in the standard mixed solution. On testing with two way ANOVA, no significant variation was observed between the results obtained for incubation periods of 25 and 30 min ($P > 0.05$). Hence, standards error (SE) of data was taken into consideration to select the optimum incubation period and 30 min with least SE (Table 4.9) was selected as the incubation period for optimal colour development.

Table 4.8: Effect of incubation periods on the sucrose level in Standard mixed solution*

Level in the aliquots of standard mixed solution (mg)	Incubation period (min)		
	25	30	35
	Estimated value (%)		
0.018	97.37 (-2.63)	100.88 (0.88)	106.75 (6.75)
0.036	98.37 (-1.63)	100.13 (0.13)	104.19 (4.19)
0.054	99.96 (-0.04)	100.88 (0.88)	104.08 (4.08)
0.072	99.66 (-0.34)	100.16 (0.16)	103.53 (3.53)
0.090	100.45 (0.45)	100.5 (0.5)	102.88 (2.88)

*Standard mixed solution contains sucrose, lactose and starch at 3, 1 and 2%.

Values in parentheses show the deviation percentage from the calculated level.

Values are mean of four trials.

Table 4.9: Statistical interpretation for incubation periods for estimation of sucrose in standard mixed solution

Treatment	Statistical Mean (mg)	Standard Error ($*10^{-3}$)
Calculated level	0.054^{ab}	------
25 min incubation	0.052^{a}	6.74
30 min incubation	0.057^{b}	6.15
35 min incubation	0.076^{c}	7.51

Number of trials: 4

Values with different superscripts vary significantly ($P < 0.05$)

4.2.2 Recovery trials

4.2.2.1 Laboratory *gulab jamun* samples (spiked with sucrose)

The colorimetric method optimized with standard mixed solution was tried with *gulab jamun* made in laboratory and spiked with known level (20-30%) of sucrose. It may be seen from the data given in Table 4.10 that the sucrose level obtained almost matched with the calculated levels of spiking. At the lowest level of spiking i.e., at 20%, the observed level was almost the same as that of the spiked level while at 30% of spiking, the level estimated was 29.6 ± 2.09%. The levels expressed on dry basis showed a similar trend.

Table 4.10: Estimation of sucrose in *gulab jamun* samples spiked with sucrose

Sucrose level (%)			
WB		DB	
Calculated	Observed	Calculated	Observed
20	20.01 ± 1.83	30.3	30 ± 2.57
25	24.66 ± 2.56	42.6	41.95 ± 2.6
30	29.6 ± 2.09	46.2	45.23 ± 1.91

Number of trials: 4

4.2.2.2 Laboratory *gulab jamun* samples (spiked with cane sugar)

As the *gulab jamun* normally is sweetened with sugar, *gulab jamun* samples spiked with cane sugar at levels ranging from 20 to 50% were estimated for sucrose content. The results are presented in Table 4.11. The levels of sucrose in these samples estimated were 20.72 ± 1.83 and 48.93 ± 1.73% at the lowest and highest levels of spiking, i.e., 20% and 50%, respectively. The levels expressed on dry basis, showed a similar trend.

Thus, it may be seen from the results that the Seliwanoff's method estimated sucrose levels almost exactly with an accuracy level of ± 3%. As may be seen from the reaction given in Figure 4.2, fructose present in sucrose is dehydrated in the presence of acid to hydroxyl methyl furfural which in turn reacts with resorcinol to give a red coloured complex with an absorption maxima at 490 nm. Since ketonic group occurs only in sucrose, the presence of lactose and starch or its hydrolytic products which are aldose-sugars in the sample filtrate or in the standard mixed solution did not interfere with the estimation of sucrose by the Seliwanoff's method.

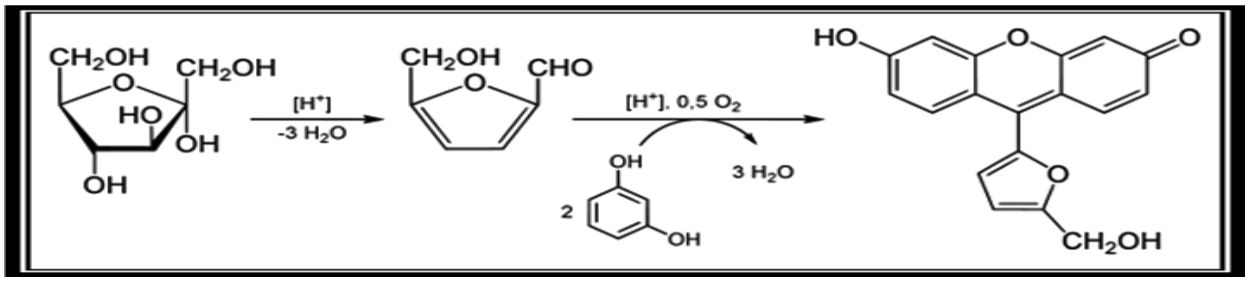


Figure 4.2: seliwanoff's colour reaction
(adopted from en.wikipedia.org/wiki/File:Seliwanow.svg)

Table 4.11: Estimation of sucrose in *gulab jamun* samples spiked with cane sugar

Sucrose level (%)			
WB		**DB**	
Calculated	**Observed**	**Calculated**	**Observed**
20	20.72 ± 1.83	37.5	36.34 ± 1.28
25	24.19 ± 1.62	41.2	40.52 ± 2.54
30	29.37 ± 1.6	49.8	48.09 ± 1.28
35	34.57 ± 2.54	52.4	51.78 ± 2.28
40	38.8 ± 1.24	58	57.79 ± 2.70
45	44.2 ± 2.11	65	64.91 ± 2.84
50	48.93 ± 1.73	69.3	68.65 ± 1.59

Number of trials: 4

4.3 Analysis of collected samples

4.3.1 Analysis of *gulab jamun* samples

The Seliwanoff's colorimetric method, optimized in the present study was used to estimate the sugar content in a few brands of market *gulab jamun* samples and the results are presented in Table 4.12. As *gulab jamun* are soaked in sugar syrup, the amount of sugar syrup adhered to the product influences the estimated level. However, the *gulab jamun* balls were freed from the adherent sugar syrup to the extent possible. It may be seen form the data presented in Table 4.12 that the sugar level in different brands of *gulab jamun* varied from 38 to 44% on wet basis and 52 to 62% on dry basis, which matched the observation of Aneja et al (2002), i.e., *gulab jamun*, contains approximately 42% sugar on drained weight basis. The method of estimation of sucrose was, however, not reported. From tabular analysis, batch-to-batch variation observed in the samples could be due to differential sugar diffusion. The sugar level in *gulab jamun* depends upon the extent of diffusion and the diffusion of sugar syrup is a function of different factors like conditions of frying, sugar syrup concentration, duration of soaking, temperature and agitation. Rao (2000) reported that the optimal sugar syrup diffusion into the *gulab jamun* balls fried at 130^{o}C for 15 min was obtained when the balls were soaked in 60% sugar syrup at 70^{o}C for 30 min with continuous stirring. Beyond 30 min, little increase in diffusion of sugar syrup was observed.

Table 4.12: Analysis of *gulab jamun* samples

Market Samples		CODE NUMBER							
		I		II		III		IV	
		WB	DB	WB	DB	WB	DB	WB	DB
		Per cent sucrose							
BATCH	1	40.8	56.8	44.4	61.6	39.8	55.2	37.9	51.8
	2	39.9	53.3	38.4	55.1	41.1	57	43.4	62.8
	3	39.5	52.3	38.1	56.4	38.9	53.3	41.5	57.1

4.3.2 Analysis of *chhana podo* samples

With the optimized Seliwanoff's colorimetric method, commercial samples of *chhana podo,* procured from the milk parlour of the Institute, were analyzed and the sugar content obtained is reported in Table 4.13. It may be seen from the tabular analysis that the sugar level in different batches of *chhana podo* varied from 24.4 to 24.8%, which was in close accordance with the value observed by Ghosh *et al.* (2007), i.e., 24.2%.

Table 4.13: Analysis of *chhana podo* samples

BATCH	Per cent sucrose	
	WB	DB
1	24.65	36.79
2	24.78	36.99
3	24.36	36.36

4.4 Analysis of dietetic sweets

A number of sweets, including milk sweets are available in the market claimed as "sugar free", "dietetic" or "diabetic" sweets. The Seliwanoff's method, optimized and evaluated in the present study was used to estimate the sugar level in these products. For this, dietetic *gulab jamun* (fully replaced with sucralose) samples were collected from the local market and dietetic *rabri* (fully or partially replaced by sucralose/ acesulfame K and aspartame) made in a laboratory of the Institute were tested for the presence of sucrose and the results are shown in Table 4.14 (a) and 4.14 (b).

4.4.1 Analysis of dietetic sweets with partial replacement of sucrose

The values of sucrose levels obtained for *rabri* with sucrose partially replaced with acesulfame K and aspartame almost matched with the calculated levels. For instance, it may be seen from Table 4.14 (a) that the estimated levels in case of

acesulfame K and aspartame were 11.23 ± 0.85 and 12.64 ± 0.17%, respectively, while the calculated levels were 11.2 and 13.7%, respectively. A similar trend was also seen when the levels was estimated on dry matter basis.

Table 4.14 (a): Analysis of Dietetic sweets*

***Rabri* with acesulfame -K**				***Rabri* with aspartame**			
Percentage sucrose				**Percentage sucrose**			
Calculated	**Observed**	**Calculated**	**Observed**	**Calculated**	**Observed**	**Calculated**	**Observed**
11.2	11.23 ± 0.85	23.09	20.86 ± 1.58	13.7	12.64 ± 0.17	25.44	26.06 ± 0.35

* Partial replacement of sucrose
Number of trials: 4

4.4.2 Analysis of dietetic sweets with full replacement of sucrose

The data given in Table 4.14 (b) shows that in case of commercial dietetic *gulab jamun*, claimed to be free from sucrose, the level of sugar was found to be very low, i.e., 2.56 ± 0.71%. Similarly, the *rabri* sample made without sugar had shown a sugar level of less than 2%. However, based on the accuracy observed in this method (±3%) with *gulab jamun* samples spiked with sugar, these dietetic samples can be considered as free from sucrose.

Table 4.14 (b): Analysis of Dietetic sweets*

***Gulab jamun* with sucralose**	***Rabri* with sucralose**
Percentage sucrose	**Percentage sucrose**
2.56 ± 0.71	1.77 ± 0.23

* Claimed as 100% sucrose replacement
Number of trials: 4

Based on the results of the study, it can be concluded that the conventional methods such as Lane-Eynon, Munson-Walker and polarimetric methods may not be suitable for estimation of sugar in milk-based sweets containing starch. On the other hand, Seliwanoff's colorimetric method, being more specific between the keto group and resorcinol, was observed to be a promising method in this regard. The optimized method was found to give better results with an accuracy of ± 3% for estimation of sugar in *gulab jamun* and *chhana podo*. When commercial samples of both the sweets were tested with the optimized colorimetric method, the values observed were in close agreement with the earlier reported value.

Chapter- 5

Summary and Conclusion

5.0 SUMMARY AND CONCLUSION

In the recent era of globalization, with increased ethnic population throughout the world, the scope for commercialization of indigenous milk - based sweets are also showing a rising trend. There is a lot of variation in the types and levels of ingredients used and methods of preparation which result in a wide variation in the physico-chemical and sensory quality of these products. In this regard, variation in sugar level appears to be the highest. In this context, estimation of sucrose content in such products is necessary to not only maintain certain quality level, but also for meeting the prerequisites of labeling and for complete compliance to standards. Total carbohydrate content of foods has, for many years, been calculated by difference, rather than analysed directly. Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of the food. This is referred to as total carbohydrate by difference and is calculated by the following formula:

100 - (Weight in grams of protein + fat + water + ash + alcohol in 100 g of food)

A number of Indian milk sweets such as *gulab jamun*, *burfi, peda, kheer, payasam* and *halwa*s are made up of milk solids, sugar and starch in the form of *maida* or *suji*. In the conventional methods (Lane-Eynon, Munson- Walker or Polarimetric method) used for determination of sucrose in milk and milk products, inversion of sucrose is done and then the difference in either reducing property or optical activity is measured as compared to lactose. However, they have not been tested for their efficiency for sugar estimation in products containing lactose and starch. In India, most of the milk sweets are either *khoa* based or *chhana* based. In this project, two sweets, viz. *gulab jamun* and *chhana podo,* which are based on *khoa* and *chhana*, respectively were chosen for evaluation of different methods for sugar estimation. These products also contain starch in addition to added sugar and milk sugar, lactose. *Gulab jamun* samples were prepared in the laboratory under controlled conditions. *Chhana podo* samples were also prepared in the Experimental Dairy plant using *chhana* made from cow milk. Samples of *gulab jamun* and *chhana podo*

(without any sugar) served as control and were tested with all the methods adopted to check their specificity for sucrose. Market samples of *gulab jamun* and *chhana podo* samples were collected from the market and the milk parlour in the Institute, respectively.

The methods adopted for evaluation were,-

A. Lane-Eynon method : SP: 18 (Part XI)- 1981
B. Munson-Walker-Bertrand's method: modified by Weinmann (1944)
C. Polarimetry : SP: 18 (Part XI)- 1981
D. Seliwanoff's colorimetric method: Pantulu *et al.* (1981)

For evaluating the above four methods, *gulab jamun* samples spiked with known amount of cane sugar (0, 30, 40 and 50%) and *chhana podo,* prepared in the Experimental Dairy plant with known sugar levels (0, 20, 30 and 40% on *chhana* basis) were taken for analysis. Samples without sugar served as the control samples. Initial trials have shown that these conventional methods (Lane- Eynon, polarimetric) were not of help for estimation of sucrose in milk sweets like *gulab jamun* and *chhana podo* probably due to the interference from the hydrolysis products of the starch formed during inversion. All the methods involved sample preparation, i.e., filtrate containing the soluble carbohydrates. In this regard, different precipitation techniques were used for precipitation and the final starch level in the filtrate depends on the method of precipitation. The methods of Lane-Eynon, Munson-Walker and polarimetry also involved inversion of sugars where degree of inversion of sugars in the presence of starch may not be consistent. Seliwanoff's method appears to help in this regard, as there is no step of inversion and more importantly, a specific reaction occurs between the keto group of sucrose and the resorcinol-HCl reagent for colour development.

Among the four methods evaluated for sucrose estimation in *gulab jamun* and *chhana podo* with known level of sugar, the colorimetric and Munson- Walker methods estimated the sugar content close to the actual level. However, considering the standard error (SE) of data, the colorimetric method appeared to be better than

Munson- Walker method regarding the consistency and repeatability. Further, Munson- Walker method is not specific to sucrose, as the control samples (without sugar) of *gulab jamun* and *chhana podo* were shown to have sugar. In this regard, Seliwanoff's method was specific and hence gave consistent results in the experimental samples of *gulab jamun* and *chhana podo*. Hence, efforts were made to optimize this method.

A standard mixed solution containing sucrose, lactose and starch in the ratio of 3:1:2, which approximately is the same that occurs *gulab jamun* was used for optimization of the method. Sucrose level in the aliquots taken for colour development was kept in the range from 0.018 to 0.090 mg as this range was observed to show a linear relation between the concentration of sucrose and the optical density. Small variation in the concentration and/ or amount of resorcinol solution did not affect the result. However, the level of the reagent was kept as 2 ml of 0.1% resorcinol solution (aqueous). Earlier studies by Pantulu *et al* (1981) showed that incubation at 70ºC was optimal for colour development. Hence, in the present study, incubation of the samples for colour development was carried out at 70ºC. In addition to temperature, incubation time may also interfere with colour development. In the present study, incubation was carried out for different periods ranging from 10 to 40 min with increments of 5 min. The initial trials in this regard showed that incubation up to 20 min was not adequate for colour development and incubation at 40 min was giving much higher values than the actual levels. Hence, incubation for 25 to 35 min was carried out for further analysis. The variation with the actual level was significant for the results obtained with incubation period of 35 min. It was seen that an incubation time of 30 min was observed to give levels of sucrose within ± 1% from the actual levels present in the standard mixed solution. On testing with two way ANOVA, no significant variation was observed between the results obtained for incubation periods of 25 and 30 min ($P > 0.05$). Hence, SE of data was taken into consideration to select the optimum incubation period and 30 min with least SE was selected as the incubation period for optimal colour development.

The colorimetric method optimized with standard mixed solution was tried with *gulab jamun* made in laboratory and spiked with known level of sucrose (20-30%) and cane sugar (20-50%). It was observed from the results that the method estimated sucrose levels almost exactly with an accuracy level of ± 3%.

The Seliwanoff's colorimetric method, optimized in the present study was used to estimate the sugar content in a few brands of market *gulab jamun* samples. As *gulab jamun* is soaked in sugar syrup, the amount of sugar syrup adhered to the product influences the estimated level. However, the *gulab jamun* balls were freed from the adherent sugar syrup to the extent possible by placing it over filter paper on a funnel for half an hour to drain out the adherent sugar syrup and then the ball was rolled over a filter paper uniformly for further removal of sugar syrup from the surface. The result showed that the sugar level in different brands of *gulab jamun* varied from 38 to 44% on wet basis and 52 to 62% on dry basis, which matched the observation of Aneja et al (2002), i.e., *gulab jamun*, contains approximately 42% sugar on drained weight basis.

When analyzed with the optimized method, the sugar level in three different batches of *chhana podo* varied from 24.4 to 24.8%, which was in close accordance with the value reported by Ghosh *et al.* (2007), i.e., 24.2%.

Dietetic *gulab jamun* (fully replaced with sucralose) samples, collected from the local market and dietetic *rabri* (fully or partially replaced by sucralose/ ascelfame K and aspartame), made in a laboratory of the Institute were tested for the presence of sucrose. The values of sucrose levels obtained for *rabri* with sucrose partially replaced with acesulfame K and aspartame almost matched with the actual levels. In case of commercial dietetic *gulab jamun* and the *rabri* sample, both claimed to be free from sucrose, the level of sugar was found to be very low, i.e., less than 2%. However, based on the accuracy observed in this method (±3%) with *gulab jamun* samples spiked with sugar, these dietetic samples can be considered as free from sucrose.

Conclusion: Based on the results of the study, it can be concluded that the conventional methods such as Lane-Eynon, Munson-Walker and polarimetric methods may not be suitable for estimation of sugar in milk based sweets containing starch. On the other hand, Seliwanoff's colorimetric method being more specific between the keto group and resorcinol, was observed to be a promising method in this regard. The optimized method was found to give better results with an accuracy of ± 3% for estimation of sugar in sweets like *gulabjamun* and *chhana podo,* containing starch along with sucrose and lactose. On testing with commercial samples of *gulabjamun* and *chhana podo*, sugar levels estimated by this method were in close agreement with earlier reported values. In diabetic sweets with partial replacement of sucrose, the level of sucrose present could be measured by this method. The colorimetric method appeared to be fast, accurate and suitable for routine analysis.

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