# SUGAR ESTIMATION-A MODIFIED APPROACH

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***Abstract :*** *The sugars are one of the major components of various food items.There are a number of well developed methods for sugar analysis which vary from simple Lane Eynon titrimetric method, polarimetric method, HPLC method with specific column like Shodex sugar column, MS methods etc. All these methods is based on aqueous solubility of sugars.However,co-extraction of proteins, polysaccharides ,lipids etc affects the validity of results. In this paper a new extraction technique using methanol is suggested and successfully applied to many food . The estimation of sugars adoptin g the aqueous method and newly developed alcoholic method is compared.*

***Keywords :*** *Sugar Analysis, AOAC methods, Polarimetry, Lane-Eynon method, Sucrose, Invert Sugar, Reducing Sugars,laddu, Methanol, Aravana etc.*

## I. INTRODUCTION

The sugarcane cultivation and sugarcane based industry can be considered as the backbone of our socio-economic system. It is the second largest agro based industry in India and plays a major role not only in Indian but also in global markets.The sugar cane based industry dates back to thousands of years as evident from the ancient literatures.

The sugars are the major components of various food items and have high analytical importance . There are many well developed specific methods for analyzing sugars in various food items.These vary from simple titrimetric to mass spectral method and HPLC methods with specific columns like Shodex sugar column .These methods are not completely applicable to varieties of applied foods even though they work well for the recommended food items like honey.This research specifically concentrates more on the sugar content analysis of Aravana - a holy sweet payasam of various Hindu temples of Kerala , Laddu-a holy sweet prasadam of Tirupati temple and other food stuff such as halwa,jilebi,Mysore pak,then sweets etc. The Aravana payasam contains many ingredients like raw rice, jaggery, black raisins, misri, cumin powder, cardamom, dry coconut pieces, dry ginger powder etc and in addition it also contains moderately high quantities of ghee. The need of a specific but rapid method for the sugar content analysis of this applied item is very much needed as the sugar content is believed to be one of the main governing parameters of the shelf life of this applied food item.

## II. MATERIALS & METHODS

### Materials:

***Study Setting*** : This study was carried out at Government Analysts' Laboratory under Commissionerate of Food Safety Trivandrum and Food Testing Laboratory,Sabarimala.

***Sample* collection :** The available sources of carbohydrate containing samples for this study includes confectioneries such as jileby ,laddu,halwa,Mysore Pak,Soan Papdi etc,locally available Kerala confectioneries such as honey sweets , poondhi , madakk boli etc and holy prasadams from temples such as Sabarimala,Tirupati,Aranmula etc.

***Apparatus and Reagents*:**

| | | |
|---|---|---|
| Electronic balance | Methanol | Fehling's Solution A and B |
| Polarimeter | Diethyl ether | Methylene Blue |
| | Chloroform | Calcium carbonate/Sodium carbonate |

Glasswares such as volumetric flask,beaker,conical flask,separatory funnel,pipette,burette etc

**Method:**

Almost all mono and oligosaccharides are water soluble and hence most of the analyzing methods extract sugars from various applied foods with water .However this extraction method has limitations like co-extraction of polysaccharides and proteins along with the targeted sugars as well as technical difficulties in filtering aqueous mixture of many foods . More over some food contain lipids and become pasty/colloidal in aqueous solution.Such methods may lead to misleading results and hence those methods have to be modified with prime importance . Almost all the polysaccharides are insoluble in hot alcohol and the methanol is used as a common precipitator of proteins . Hence a method developed based on these two properties viz. aqueous solubility and the capacity of methanol to precipitate proteins can be used to overcome the present hurdles.

**Experimental :**

**PRESENT METHOD** : The present estimation method is based on solubility of sugars in water .The method is briefly explained as follows:

1. Sample is homogenized
2. A known quantity of sample is weighed accurately into a beaker,dissolve in distilled water and make upto volume.

3. This solution is used for the detection of total free reducing sugar(FRS) in the sample.
4. A known volume of the sample solution is inverted for the determination of total sugar as invert sugar(TS).
5. Sucrose is calculated by the difference multiplied with invert factor

These are calculated by the equation

$$\textbf{FRS or TS} = \frac{\textbf{Fehling's factor x Dilution x 100}}{\textbf{Weight of sample x Titre value}}$$

$$\textbf{Sucrose} = \textbf{(TS – FRS) x 0.95}$$

**PROPOSED METHOD**: The proposed method is based on methanol extraction.

1. Sample is homogenized
2. A known quantity of the sample weighed accurately is into a dry conical flask.
3. The food samples may contain lipids,which should be removed prior to alcohol extraction.The sample is mixed with solvents like diethyl ether or hexane,shake occasionally,decant lipid containing solvent and residue evaporate on a water bath.
4. Some food samples may be partially acidic.Samples are neutralized before alcohol extraction to prevent hydrolysis of sucrose and starch.This is done by adding 2-3 grams of calcium carbonate or sodium carbonate
5. The lipid free and neutral sample is extracted successively with 80%, 75% and 70% aqueous methanol by refluxing on a water bath at $60^{O}$C for 5-10 minutes.The completion of sugar extraction can be tested by washing the residue with deionized water until Molisch test is negative
6. The combined supernatant is collected in a separatory funnel and extracted with chloroform (1:1).
7. The upper alcohol layer is collected and alcohol is evaporated off by placing over a water bath maintained at 65 $^{O}$C.
8. The aqueous concentrate is cooled and diluted to 100 ml with deionized water . This solution is used for detecting free reducing sugar in the sample.
9. A known volume of this sample solution is inverted for the determination of total sugar as invert sugar.
10. Sucrose is calculated by the difference multiplied with the invert factor.

The methanol extract is also analysed polarimetrically.In some cases clarification is required. Polarimetrically ,sucrose and invert sugar are calculated by the equations

$$\textbf{Sucrose} = \frac{\textbf{(D-I) X 100}}{\textbf{132.56 + 0.0794 x weight correction}}$$

$$\textbf{Invert sugar} = \frac{\textbf{66.5 X (D – sucrose)}}{\textbf{-20}}$$

Where **D** is the direct polarimetric reading , **I** is the total invert reading which is obtained by multiplying original invert reading with a suitable factor; For example if 20 ml sample solution is inverted,the invert reading should be multiplied by 5 to account for actual weight of sample;similarly , reading is multiplied with 4 if 25 ml solution is inverted because initially sample is make up to 100 ml. , **weight correction**- suppose 20 ml of 100 ml solution of half normal weight (13 g) of sample is inverted,the inverted solution contains only 2.6 grams of sample.If actual weight of sample is 13.5420 grams , then 20 ml inverted solution contains 2.7084 grams sample.Therefore weight correction is 2.7084-2.6 = 0.1084.

**Note**: The normal weight of sugar product for analysis using polarimetric tube of 200 mm is 26 grams.But our analysis is conducted in RUDOLPH RESEARCH ANALYTICAL AUTOPOL 1 automatic polarimeter with 100 mm tube.Hence we used 13 grams of sample.

## III. RESULTS

The samples collected were analyzed using both the aqueous and alcoholic methods. Some samples can't be analysed by aqueous method (eg.jilebi,laddu…) . The samples added with the known quantities of sugar were also analyzed using both the methods to compare the validity of the proposed method.The results are tabulated for better understanding.

| Sl No | Item analyzed | Free Reducing Sugar (% by mass) | | Total Sugar as Invert Sugar (% by mass) | | Sucrose content (% by mass) | |
|---|---|---|---|---|---|---|---|
| | | Present Method | Proposed Method | Present Method | Proposed Method | Present Method | Proposed Method |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | Aravana Prasadam Sabarimala Temple<br>Batch No.37 | 11.75 | 6.74 | 62.61 | 59.45 | 48.32 | 50.07 |
| 2 | Aravana Prasadam Sabarimala Temple<br>Batch No.64 | 10.84 | 4.05 | 60.35 | 55.67 | 47.03 | 49.04 |
| 3 | Aravana Prasadam Sabarimala Temple<br>Batch No.65 | 9.96 | 5.61 | 59.72 | 56.46 | 47.27 | 48.31 |
| 4 | Aravana Prasadam Aranmula Temple | 8.76 | 3.17 | 50.85 | 47.94 | 39.99 | 42.53 |
| 5 | Aravana Prasadam Puthiyakavu Temple Kollam | 7.19 | 0.74 | 49.68 | 44.90 | 40.37 | 41.95 |
| 6 | Laddu Prasadam<br>Tirupati Temple | -- | 0.79 | -- | 41.99 | -- | 39.14 |
| 7 | Halwa Black Kozhikkodan | -- | 3.85 | -- | 40.12 | -- | 34.46 |
| 8 | Halwa<br>Red Coloured | -- | 10.01 | -- | 34.58 | -- | 23.34 |
| 9 | Halwa<br>Black Coloured | -- | 4.48 | -- | 24.26 | -- | 18.79 |
| 10 | Jilebi<br>Orange Coloured | -- | 2.53 | -- | 27.69 | -- | 23.90 |
| 11 | Jilebi<br>Orange Coloured | -- | 2.0 | -- | 39.51 | -- | 35.63 |
| 12 | Laddu<br>Orange Coloured | -- | 3.51 | -- | 34.52 | -- | 29.46 |
| 13 | Laddu<br>Yellow Coloured | -- | 1.20 | -- | 40.38 | -- | 37.22 |
| 14 | Honey Sweets<br>(Then sweets) | -- | Negligible | -- | 39.0 | -- | 37.05 |
| 15 | Laddu<br>Orange Coloured | -- | 4.07 | -- | 32.98 | -- | 26.96 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 16 | Item no.15 with added known quantity of sugar (2.1009 g cane sugar of 98.38 % sucrose) | -- | 4.60 | -- | 34.62 | -- | 29.02 |
| 17 | Item no.15 with added known quantity of sugar (3.8364 g cane sugar of 98.38 % sucrose) | -- | 4.19 | -- | 36.42 | -- | 30.62 |
| 18 | Mysore pak<br>Yellow Coloured | -- | 3.02 | -- | 38.76 | -- | 33.95 |
| 19 | Mysore Pak<br>Light Orange Coloured | -- | 3.61 | -- | 40.23 | | 34.79 |
| 20 | Soan Papdi | -- | 2.98 | -- | 39.05 | -- | 34.27 |
| 21 | Kesari Pudding | -- | 3.49 | -- | 46.17 | -- | 40.55 |
| 22 | Aravana Prasadam Sabarimala Temple<br>Batch No.58 | 11.03 | 5.86 | 63.17 | 61.24 | 49.53 | 52.61 |
| 23 | Item no.22 with added known quantity of sugar<br>(4.1275 g cane sugar of 99.03 % sucrose) | 10.64 | -- | 66.25 | --- | 52.83 | -- |
| 24 | Item no.22 with added known quantity of sugar<br>(3.9572 g cane sugar of 99.03 % sucrose) | -- | 5.84 | -- | 65.29 | -- | 56.48 |
| 25 | Poondhi | -- | 2.94 | -- | 34.62 | -- | 30.10 |
| 26 | Madakk Boli | -- | 3.06 | -- | 31.85 | -- | 27.35 |
| 27 | Chewing Gum | -- | 5.03 | -- | 63.32 | -- | 55.38 |
| 28 | Jilebi<br>Orange Coloured | -- | 2.56 | -- | 31.72 | -- | 27.70 |

Some samples are also analysed polarimetrically to check the fitness of this method . The results of polarimetric and titrimetric analysis are given in the following table.

| Sl No | Item analysed | Titrimetry | | Polarimetry | |
|---|---|---|---|---|---|
| | | Sucrose (% by mass) | Invert Sugar(% by mass) | Sucrose (% by mass) | Invert Sugar (% by mass) |
| 1 | Aravana Prasadam Sabarimala Temple Batch No.37 | 50.07 | 59.45 | 50.12 | 59.98 |
| 2 | Laddu Prasadam Tirupati Temple | 39.14 | 41.99 | 40.05 | 42.92 |
| 3 | Halwa Black Kozhikkodan | 34.46 | 40.12 | 34.82 | 40.36 |
| 4 | Halwa Black Coloured | 18.79 | 24.26 | 19.16 | 24.60 |
| 5 | Chewing Gum | 55.38 | 63.32 | 56.27 | 64.24 |
| 6 | Jilebi Orange Coloured | 35.63 | 39.51 | 35.71 | 39.30 |
| 7 | Laddu Yellow Coloured | 37.22 | 40.38 | 37.46 | 40.56 |
| 8 | Jilebi Orange Coloured | 27.70 | 31.72 | 28.07 | 32.12 |
| 9 | Aravana Prasadam Aranmula Temple | 42.53 | 47.94 | 42.84 | 48.08 |
| 10 | Halwa Red Coloured | 23.34 | 34.58 | 23.47 | 34.61 |

The detailed calculation for laddu prasadam of Tirupati Temple is given as reference.

Sample Name : **Laddu from Tirupati temple** Date of analysis : 27.05.2019

Weight of sample = 14.1328 g
Methanol extraction and make up to 100 ml.Invert 25 ml of this solution and make up to 100ml

**Polarimetry**

Direct reading = 27.14
Invert reading = - 6.49 Total invert reading = - 6.49 x 4 = - 25.96

$$\text{Sucrose} = \frac{(27.14 - -25.96) \times 100}{132.56 + 0.0794 \times (3.5332-3.25)} = 40.051289 = 40.05\ \%$$

$$\text{Invert sugar} = \frac{66.5 \times (27.14-40.05)}{-20} = 42.92575 = 42.92\ \%$$

**Titrimetry**

Titre value for FRS = 44.9 ml

$$\text{FRS} = \frac{0.05044 \times 100 \times 100}{14.1328 \times 44.9} = 0.794878 = 0.79\ \%$$

Titre value for TS = 3.4 ml

$$TS = \frac{0.05044 \times 100 \times 100 \times 100}{13.0384 \times 20 \times 3.4} = 41.988 = 41.99\ \%$$

$$\text{Sucrose} = (TS - FRS) \times 0.95 = (41.99 - 0.79) \times 0.95 = 39.14\ \%$$

## IV. DISCUSSION

The alcoholic method was found more suitable for estimation of sugars with many superior characteristics. It is find very difficult to extract sugar from most food using water ; it is possible for some food like Aravana, but the aqueous method provides high value of free reducing sugar and total sugar as invert sugar but relatively low sucrose as compared with methanol method .This is probably because of the dissolution of amylose component of starch present in food ,its break up in acidic solution to glucose which usually increase the percentage of reducing sugars .The acidity of food also cause inversion of some sucrose which further enhance the reducing sugar content .These factors lead to low sucrose content of food product whose percentage actually determines the shelf life of food products in the absence of added preservatives .In the methanol extraction method,starch remains insoluble in methanol and acidity is removed before extraction by adding weak base like sodium or calcium carbonate to block the inversion of sucrose and breakage of starch (amylose) during extraction process so that exact amounts of free reducing sugar, invert sugar and sucrose is obtained. The results obtained by the proposed methanol extraction method were found more accurate than the presently adopted aqueous method for sugar estimation . The methanolic extracts were analyzed both titrimetrically and polarimetrically.The results are in good agreement.

The calculated quantities of sugar added during the preparation of food and those food samples were also subjected to the analysis for validating the developed method . The percentage recovery of added known quantity of sugar is excellent in the proposed method.

## V. CONCLUSION

The developed method has successfully applied to many food samples for estimating the sugar content more accurately. This method was found superior to the present method as it can successfully manage the issues like co-extraction as well as the filtration difficulties and can be recommended to estimate the sugar contents in applied products.

## VI. REFERNCES

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