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Reducing Sugar Determination of Jaggery by Classical Lane and Eynon Method & 3, 5-Dinitrosalicylic Acid Method

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Abstract: *Jaggery is a sweetener consumed by many people in different parts of the world and carbohydrates are one of the most essential component of jaggery or any other food which can be determined by knowing the reducing sugar. In other sense, reducing sugar is the chemical term of sugar that acts as a reducing agent because it has free aldehyde and ketone functional group. Since jaggery is an unrefined product with rich nutritional and medicinal values as compared to sugar, it becomes necessary to determine the presence of reducing sugar of jaggery in order to determine its composition. A comparison has been made between the classical Lane and Eynon (Titration) method & 3,5- Dinitrosalicylic Acid (DNSA) method to determine the reducing sugar (RS).The classical lane and Eynon method for assaying of total sugar content in food is based on copper reduction method before and after inversion and is well known in sugar industry. DNSA method was first introduced by Sumner in 1921 for assaying reducing sugars in urine and later on Sumner and Howell in 1935 adapted this method to determine reducing sugars for different food products. Four different jaggery samples viz Shud Sattvic, Organic Tattva, Terra Green and Kolhapur jaggery were used for assaying reducing sugar. The results obtained by both these 7-8methods show no statistical difference between the values of RS.*

1. Introduction

Jaggery is a traditional sweetener which is produced in addition of refined sugar from sugarcane [1-2]. 100g of Jaggery contains approximately 65 to 85g sucrose, 5 to 15g glucose and fructose. Along with 0.4g of protein, 0.1g of fat and 0.6-1g of minerals, (viz; 0.008g calcium, 0.004g of Phosphorus, 0.011g iron, 0.070-0.090g of Magnesium, 1.05gm of Potassium, minerals like Manganese, Zinc, Copper and Chloride are also present in small amount.) [3]. According to the Ayurveda, Jaggery is considered as a base material for the preparation of medicines. Jaggery contains 65-85% sucrose along with the mineral content, whereas the white crystal sugar contains only sucrose with 99.5% without any minerals [4].

Estimation of reducing sugar is useful in identifying the presence of carbohydrate as well to identify grade and composition in jaggery [5]. Carbohydrates can be classified into three groups, where first group consists of sugar, then oligosaccharides and lastly polysaccharides. Where sugar further can classified as monosaccharaides, disaccharides and polyols. Monosaccharaides are water soluble crystalline compound. They are aliphatic aldehydes and ketone which contain one carbonyl group and one or more hydroxyl group. Oligosaccharides are low molecular weight polymers of monosaccharaides (<20) that are covalently bonded to glycosidic linkage. Majority of the carbohydrates found in nature are present as polysaccharides, these are high molecular weight polymer of monosaccharaides (>20) which will show the presence of free aldehyde and ketone functional group [6]. A reducing sugar is any sugar which can act as reducing agent because of free aldehyde and ketone group. All monosaccharaides, along with some disaccharides, oligosaccharides and polysaccharides are reducing sugar. The common dietary monosaccharaides like glucose, fructose and galactose are reducing sugar whereas sucrose acts as non-reducing sugar.

Reducing sugar of jaggery or any other compound can be determined by various methods such as chromatography [7-8] and electrophoretic methods [9-10], Titration Method [11-12], Gravimetric method [13-14], Colorimetric [15-16], and UV [17-18] etc. The current investigation focuses on the determination of Reducing Sugar of jaggery samples of the brand name such as Shud Sattvic, Organic Tattva, Terra Green and the jaggery obtained from local manufacturing company in Kolhapur by Classical Lane and Eynon (Titration) Method and 3,5 Dinitrosalicylic Acid (DNSA)

method. The Lane and Eynon method for determination of total sugar content in Jaggery is based on copper reduction method before and after inversion (Horowitz, 1980). Lane and Eynon method (titration method) is simple and well known in sugar industry and doesn't require any expensive instrumentation. Titration method is based on empirically derived constants and excess acid or excess heat may cause errors in the procedure [19]. Titration method requires jaggery samples which are mixed with lead acetate (clarificant), potassium oxalate & Sodium phosphate. The solution is titrated against Fehling Solution (Fehling A [$CuSO_4 5H_2O$] Crystals and Fehling B [$KNaC_4H4O_6$] i.e. Rochelle salt or Potassium Sodium Tartrate) which was heated at 103^0 to 106^0 Celsius. Lane and Eynon method is strongly depend on the boiling condition of solution (ICUMSA, 1970), therefore the time taken to reach the boiling point should be standardized. Several procedures state that the solution to be titrated should come to boil in 2 to 2.5minutes [20]. Significant deviation in titre may show little error in result.

To crosscheck and verify the results derived from Lane and Eynon method, experiments for determination of reducing sugar were performed with3, 5 Dinitrosalicylic acid and under UV visible spectroscopy. This method involves uniform mixing of the sample (Jaggery) with DNSA reagent, heating to catalyze the reaction (equation I) and measuring the visible absorbance of the product.

3, 5 Dinitrosalicylic acid + Reducing Sugar $\longrightarrow$
3-Amino-5-nitrosalicylic acid + Oxidised Sugar
- - - - (I)

Sumner JB, 1924, proposed a reaction based on which the reducing sugar in different kind of foods and beverages was made possible. DNSA method is based on two aspects; one is oxidation of functional sugar groups present and second is the reduction of DNSA to 3-amino 5-nitrosalicylic acid based on the alkaline conditions present and heat, which absorbs light at 540nm [21]. The addition of phenol and sodium bisulfite along with sodium hydroxide used in this method (Sumner, 1925) required for redox reaction to occur between DNSA and reducing sugar.

2. Materials and Methods

A] Analysis of Reducing Sugar by Lane and Eynon Method:

The solution of 10% w/v lead acetate trihydrate (99%) reagent was prepared as follows: 10gm of lead acetate trihydrate was dissolved to a final volume of 250ml distilled water. A 10% w/v control solution of di-potassium oxalate monohydrate and di-sodium hydrogen phosphate anhydrous was prepared as follows: 17.5gm of di-potassium oxalate monohydrate along with 7.5gm of di-sodium hydrogen phosphate was dissolved in distilled water and make up was done to 250ml was in volumetric flask to obtain high precision [12]. 10% w/v solution was prepared by dissolving 10gm of jaggery sample using a beaker and a magnetic stirrer on a hot plate. This solution was made up to 100ml in volumetric flask of which 75ml was taken in a measuring cylinder and 10ml of each solution of lead acetate trihydrate and solution of di-potassium oxalate monohydrate and di-sodium hydrogen phosphate was added. 75ml of jaggery solution thus prepared was made up to 250ml. This solution was later filtered using whatman 42 filter paper. 5 ml of each Fehling's solution A & of Fehling's solution B was taken in Erlenmeyer flask and placed in heating mantle until boils and 2-3 drops of methylene blue indicator was added. This was then titrated using filtrate obtained as the burette solution till end point was brick red. The endpoint was confirmed by adding a drop of methylene blue indicator and, if the blue color disappeared [22], it meant the experiment performed was correct and the reading on the burette was thus noted. This experimental procedure was followed for each jaggery sample.

From the experiments performed by titration method, the reducing sugar of various jaggery samples namely Shud Sattvic, Organic Tattva, Terra Green and Kolhapur jaggery sample was calculated by the equation no II

$$\% \text{ Reducing Sugar (RS)} = \left[\frac{X}{B.R} * \text{Dilution Factor}\right] * 10 \quad \text{- - - - (II)}$$

$$\text{Dilution Factor} = \left[\frac{\text{sample volume}}{250}\right] \quad \text{- - - - (III)}$$

Where,
X - Constant factor calculated from the known RS.
B.R – Burette reading.
Dilution factor was calculated using equation no III.

B] Analysis of Reducing Sugar by DNSA method

The dinitrosalicylic acid method uses the following chemicals: Dinitrosalicylic acid, Rochelle salt, phenol, sodium bisulfite and sodium hydroxide. The IUPAC recommended DNS reagent, containing phenol and sodium metabisulfite was prepared as follows: 10gm of di-nitro salicylic acid, 10gm of sodium hydroxide, 300gm of Rochelle Salts (potassium sodium tartrate), 2gm of phenol crystals melted at 50°C and 0.5gm of sodium metabisulfite

were dissolved to a final volume of 100ml with distilled water [23].

Varying concentrations of standard jaggery solution viz; 0-0.8 mg/ml were prepared for four jaggery samples. 3ml aliquots of each standard solution of jaggery prepared were added to the different test tubes and to this were added 3ml aliquots of DNS reagent. The test tubes were covered to avoid the loss of liquid due to evaporation. The reaction mixtures in the test tubes were heated in a boiling water bath for 5-15 minutes to develop red brown color. Add 1ml of 40% of Rochelle salts (potassium sodium tartrate) solution to the reaction mixture subsequent to development of color and prior to cooling. The test tubes were cooled to room temperature in a cold water bath. Record the absorbance at 540nm with spectrophotometer [24]. Figure 1 depicts the above mentioned experimental procedure.

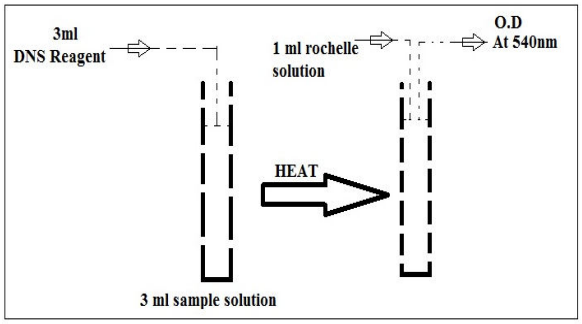

Figure1. Experimental Setup for DNSA method

Plot the Calibration curve for absorbance v/s concentration at 540nm as shown in figure 2. The average reading obtained from UV-spectroscopy and the line equation thus obtained, helps calculate the % reducing sugar of the jaggery samples.

3. Results and Discussion

The presence of reducing ends in the foods and the measurements of the concentration of reducing ends produced valuable information about the sample to be analyzed. Reducing sugar have gained considerable importance which lead to a growing number of methods for their assay [25]. The level of reducing sugar helps determine the quality of jaggery and its constant monitoring during the production has improved the market quality.

The lane and Eynon method and 3,5 Dinitrosalicylic acid method was used in the determination of reducing sugar % content of the jaggery samples. Table 1 summarizes the results of the determination of reducing sugar % of various jaggery samples by lane and Eynon method.

Table 1. Results by Titration Method.

Sample Name	Average Burette Reading	% Reducing Sugar
Shud Sattvic	15.9	10.75
Organic Tattva	13.45	12.71
Terra Green	34.35	4.98
Kolhapur	32.9	5.20

A method is described for the quantitative estimation of reducing sugar content of jaggery by titrimetric method containing lead acetate, potassium oxalate, sodium hydrogen phosphate, Fehling's A and B solution and methylene blue indicator. The reducing sugar of Shud Sattvic, Organic Tattva was found greater (10.75% and 12.71% respectively) compared to Terra Green and jaggery obtained from local manufacturer in Kolhapur (4.98% and 5.20% respectively). The experimental procedure followed in this paper for the determination of reducing sugar was also pursued by Balagat C. et al for maple syrup (27.01%), Muhammad Shahnawaz et al for Jamun fruit products like jam (27.43%), squash (18.27%), ready to drink juice (7.42%), seed powder (1.22%) and pulp powder (3.70%) and Jesus Manuel Ramon Sierra et al for honeys of Apis melliferra (69.2%), Melipona beecheii (53.1%), and Trigona spp (63.7%).

Henry Lane and Lewis Eynon proposed the use of methylene blue as an indicator for Fehling's solution for the exact volumetric determination of the more important reducing sugars. Fehling's A comprises of copper sulphate in distilled water which is deep blue and Fehling's B is a colorless solution of Rochelle salt (potassium sodium tartrate) and sodium hydroxide in distilled water. Fehling's reagent was used as they give a positive result for aldose and ketose monosaccharaides. Upon the addition of Fehling's, the blue copper(II) ions will be reduced to copper(I) ions which precipitates out of the solution as red copper(I) oxide and will help determine the amount of sugar by titration . Lead acetate was used to obtain sharper end points and is widely used for removal of coarse dispersoids, thus used for clarification. Potassium oxalate reagent was used to remove excess of lead from the defecated liquid [26]. Di sodium hydrogen phosphate accelerates the rate of oxidation of same hexoses with air. The decrease in the total reducing sugar of the solution was decided by the action of di sodium hydrogen phosphate on glucose and fructose [27].

Table 2 elicits the results of the determination of reducing sugar % of various jaggery samples by DNSA method. The DNS assay was first discovered for its use in the measuring of sugars in the urine and blood [21, 27]. DNSA is the alkaline 3, 5

Dinitrosalicylic acid method of Miller has been shown to be relatively faster and convenient. The reducing sugar % was calculated from the calibration of plot of absorbance v/s concentration at λmax as shown in the figure.

Table 2. Results by DNSA Method.

Sample Name	Average Reading	X	% Reducing Sugar
Shud Sattvic	0.61	0.19	9.56
Organic Tattva	0.73	0.23	11.28
Terra Green	0.30	0.10	4.89
Kolhapur	0.31	0.11	5.05

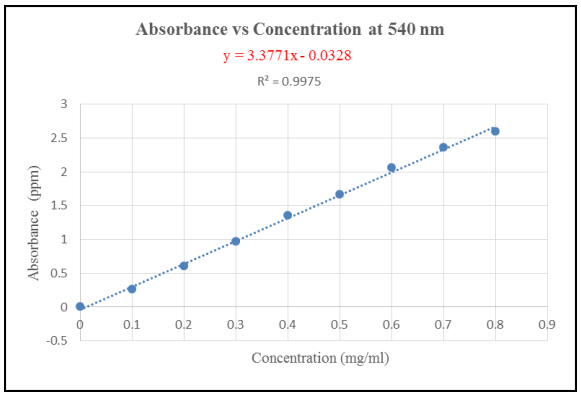

Figure2. Absorbance vs Concentration

The DNS reagent prepared according to Miller was subsequently improved by the addition of Rochelle salt (sodium potassium tartrate) which prevents the reagent from dissolved oxygen by reducing the dissolved oxygen by the ion concentration in the solution [29]. Later in 1925, Sumner proposed the addition of phenol and sodium meta bisulfite. Though phenol has no reducing action upon Dinitrosalicylic acid, it enhances the colour intensity produced and counteracts the effect of phenol compounds present [30]. Sodium meta bisulfite which reacts with oxygen was used to stabilise the colour in presence of phenol. Sodium hydroxide, an alkaline medium is required for redox reaction to occur between DNS and the reducing sugars [21, 23]. DNS reagent composition and the sugar assay method were prepared according to the work published by Miller.

Results obtained by DNSA method for reducing sugar of Shud Sattvic and Organic Tattva (9.56% and 11.28% respectively) were high compared to Terra Green and the jaggery obtained from local manufacturer in Kolhapur (4.89% and 5.05% respectively). The reducing sugar content of 5 brands of unifloral honey namely litchi, mustard, plum, til and kadom was found in the range of 62.30-65.02% using dinitrosalicylic method [31].

4. Conclusion

The results obtained by the two proposed methods namely, Lane and Eynon (titration method) and 3,5-Dinitrosalicylic acid (DNSA method) are in the range of 4.98% to 12.71%and 4.89% to 11.28% respectively. It was observed that the % reducing sugar of each sample obtained by both the methods was approximately similar and the analysis thus help in finding the composition of jaggery.

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