Chemistry and Materials Research
ISSN 2224- 3224 (Print) ISSN 2225- 0956 (Online)
Vol.6 No.8, 2014 

# Relative Examination of Monosaccharide and Disaccharide Adopting Polarimeter and UV-Visible Spectrophotometer Instruments.

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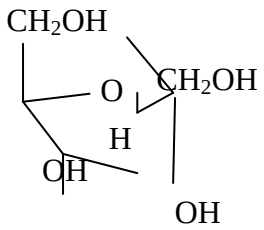
**Abstract**
Polarimeter and ultra violet-visible spectrophotometer instruments were used to analyze monosaccharide and disaccharide. Linear regression equation and analysis of variance (ANOVA) were applied for statistical analysis. Uv-visible spectrophotometer was more sensitive than polarimeter ($R^2$ = 0.94). Significant difference ($P \leq 0.05$) was observed between the two methods. Identification of higher percentage of specific monosaccharides was possible. This was shown by the significant difference among sugars for the methods. Uv-visible spectrophotometer and polarimetry showed least sensitivity for determination of fructose. In recent times, ethanol from bio resource is being considered as an alternative source of energy, glucose which is one of the monosaccharide of carbohydrate can be converted to ethanol, and the quantity of the ethanol produced is dependent on the initial concentration of (carbohydrate) that was fermented. Other industrial use of carbohydrates includes pharmaceutical uses, paints ink etc. It is therefore important to compare instruments which will be adopted for rapid, sensitive and accurate results on the quantity and quality of carbohydrates.
**Keywords:** Monosaccharide, disaccharide, Uv-spectrophotometer and Polarimeter.

## Introduction

Monosaccharide is the simplest form of carbohydrate. They consist of one sugar and are usually colorless, water-soluble, crystalline solids. Some monosaccharide glucose (dextrose), fructose, galactose, and ribose.Monosaccharide are the building block of disaccharide like sucrose (common sugar) and polysaccharides (such as cellulose and starch) (Wikipedia.org/wiki retrieve 2012)




The reactivecentres of monosaccharide are carbonyl and hydroxylgroups.
Oligosaccharide isrelatively low molecular weight polymers of monosaccharides (< 20) that are covalently bonded through glycosidic linkages.Oligosaccharides containing glucose, fructose and galactose monomers are the most commonly occurring in food. Many analytical techniques have been used to measure the total concentration and type of carbohydrates presents in foods, industrial starch and additives in various muds. Some of the techniques includes, chemical methods using titration, gravimetric methods, colorimetric, enzymatic and physical methods (McClement et al 2009)
The physical methods include polarimetry, refractive index (refractometry) density and infrared. Immunoassays are finding increasing use in the food industry for qualitative and quantitative analysis of low molecular weight carbohydrate.
Processing sample for carbohydrate analysis depends on the substance being analyzed. The precise method of carbohydrate isolation depends on the carbohydrate type, the matrix type and purpose of analysis. However there are some procedures that are common to many isolation techniques. For example substances containing carbohydrates are usually dried under vacuum (to prevent thermal degradation), ground to a fine powder (to enhance solvent extraction) and then defected by solvent extraction.
Boiling a defiled sample with an 80% ethanol solution is one of the most commonly used methods of extracting low molecular weight carbohydrate from substances. Monosaccharides and disaccharides (oligosaccharides) are soluble in alcoholic solution, whereas, proteins, polysaccharides and dietryfibre are insoluble. Various other molecules present in alcoholic extract must be removed before carbohydrate analysis. This is commonly

achieved by treating the solution with clarifying agent like heavy metal salts such as lead acetate or by passing it through one or more ion-exchange resins .

The concentration of carbohydrate can be determined gravimetrically, spectrophotometrically or by titration. Non-recluding carbohydrate can be determined using the same methods, if they are first hydrolysed to make them reducing.

Anthrone method is an example of a colorimetric method of determining the concentration of the total sugars in a sample . Colorimetric method has been used in the work of (Springerlink.com/index/c11412 2011)

Most of the methods are non-stoichiometric. Therefore it is necessary to prepare a calibration curve using a series of standards of known carbohydrate concentration (Association of Analytical Chemists AOAC,1980).Many different physical methods have been used to determine the carbohydrate concentration of substances. Physical methods of analysis rely on change in some physicochemical characteristic of a carbohydrate as its concentration varies.

Cellulose and hemicelluloses are normally reduced to monosaccharide in the conversion of biomass to energy. Only glucose is useful in the conversion to ethanol which is a petrol blending component. It is necessary to determine the percentage of glucose in the cellulose hydrolysate formed. This determination is not an easy task. Shortly before the present the introduction of ion exchange chromatographic columns with pulsed amperometric detector for high performance liquid chromatograph, HPLC has facilitated this discriminative analysis. This facility as at present is a technology not available in Nigeria.

In view of this, it is important to use other methods of monosaccharide/disaccharide determination such aspolarimetry and µv-visible spectrophotometry which are available in the country for concentration determination. Optimization of the procedure for more sensitive results will also be investigated. This study is essential as it will make use of the available instruments around us.Some past work carried out so far showed that sugars are subdivided into two major groups of Monosaccharide and disaccharide (Oligosaccharide) (I.L, Finar 1973) Polysaccharides are more complex than the sugars, the molecule weights being for greater.

Glucose is one of the examples of monosaccharide that can be found in rite grapes, honey and sweet fruits. It is also a normal constituent of blood and occurs in the urine of diabetic patient. Glucose is a product of dilute acid hydrolysis of hemicellulose at temperature of 200 – 220$^{O}$C.

Ethanol is produced from hydrolysis of glucose obtained from the hemicellulose of biomass. (Millati et al 2005,Brandberg et al 2005, Purwadi et al 2004) showed that batch reactors have been the most widely used reactors for kinetic study of hydrolysis in laboratory and pilot study for ethanol production obtained from Lignocelluloses materials.

Sucrose is one of the disaccharides that are crystalline, solids, and soluble in water. Sucrose, cane-sugar is one of the most important compoundscommercially and is obtained from the sugar cane and sugar-beet. In addition to crystalline sucrose syrup is always obtained which will not crystallize. This syrup, known as molasses, is also commercial product.

The optimal conditions of steam explosion pretreatment of sugarcane bagasse of biomass have been found to be as follows: 220$^{O}$C, 30 seconds residence time, water – to – solids ratio, 2:1 and 1% $H_2SO_4$ (Morjanoff, and Gray 1987)

Ammonia fibre explosion, AFEX, is a type of Physico-chemical pretreatment in which lignocelluloses materials are exposed to liquid ammonia at high temperature and pressure for a period of terms and the pressure swiftly reduced.

AFEX pretreatment can be used for the pretreatment of many ligonocellulosic materials including sugar cane bagasse (HoltZapple et al 1991).

Some researchers have used carbon dioxide explosion method to pretreat biomass containing sugar cane (4kg $CO_2$/kg fibre at the pressure of 5.62Mpa and obtained 75% of the theoretical glucose obtained during 24hrs of enzymatic hydrolysis (Dale 1980)

Evaluations of these components have been carried out using polarimetry and Uv-visible spectrometer. Several workers have used some of these instruments. But in this case weused two instrumentspolarimeter and µv-spectrophotometer at the same time for the same group of substances. Novel practical method was used to compare the immunoglobine concentrations of a number of animals applying sugar, and alcohol refractometers, colostromele and spectrophotometer. Measurements obtained with the two refractometer were correlated (R = 0.99) and highly reproducible (R = 0.98) and (R = 0.99) for the sugar and alcohol refractometers, respectively (Chavatte et al 1998)

The colostrometer measurement were less reproducibly (R = 0.61). optical density showed no relationship to immunoglobin concentrations or plasma immunoglobin levels. Polarimeter was however not used by their workers (Chavatte et al 1998)

(Hassani et al 2009)Compare sugar analysis methods in Penicillin-G fermentation broth they observed that although the refractometry is very simple in sugar analysis, however, existence of various amino acids and other optically active materials such as corn steep liquor (CSL) and molasses can affect on the total sugar

concentration in broth whichcould lower accuracy of measured results. They also observed that althoughcolorimetic method showed good results, that accuracy of the colorimetric method was decreased with decreasing of sugar concentration in the final steps of fermentation.Sugar industry with its international commission for uniformmethods of sugar analysis (ICUMSA) introduces international sugar scale (ISS) in $^{O}Z$ unit $100^{O}z$ unit (sugar degrees) belong to normal sucrose solution prepared from exactly 26g of sucrose dissolved in pure water to $100^{O}cm$.

## MATERIALS AND METHODS

Pure substances of glucose, fructose, sucrose, maltose and lactose were used in this study. Polarimetry model D polarimetric Bellingham and stanlly limited, England Polarimter tube Jenway 6305 andUv-spectrometer used were obtained from federal university of techcnologyOwerri,Imo state. All standard solutions of these pure samples were prepared.

A standard solution of the mixture was also prepared by mixing equal weights of glucose, fructose, sucrose, maltose and lactose.

### Methods

The concentration used were 2%,4%, 6%, 8%, 10%, 15%, and 20%. The preparation for each of the individual standard solution was done as follows: for 2%,4%, 6%, 8%, 10%, 15%, and 20%: 1g, 2g, 3g, 4g, 5g, 7.5g, and 10g of each standard sugars were dissolved in $50cm^3$ of solution with distilled water respectively.

The standard solution mixtures were also prepared by using 0.2g, 0.4g, 0.6g, 0.8g, 1.0g, 1.5g, and 2.0g of each sugars glucose, fructose, sucrose, maltose and lactose were mixed and dissolved in $50cm^3$ of solution with distilled water for 4%, 6%, 8%, 10%, 15%, and 20% respectively.

Each sugar standard including the standard mixtures had seven different concentrationsof 2%, 4%, 6%, 8%, 10%, 15%, and 20% wereanalysed using polarimetry instruments. The value of the angle through which the plane of polarized light has been rotated was read directly from the scale. The reading for the standard machine was also obtained in a similar manner.

Seven different concentrations of prepared samples of standard solutions and standard mixtures were also analyzed using µv-visible spectrophotometer. The absorbance readings of the different concentration of each sugar standard and the standard mixture were taken in turn at 490nm (wavelength).

The data collected from the analysis were treated using linear regression and analysis of variance, (ANOVA) all statistical analysis was at 95% confidence limit, $P \leq 0.05$. The results were presented in graphs figures while the statistical treatments were presented in tables.

## RESULTS AND DISCUSSION

Table 1 result: reading obtained with various sugar standard and standard mixture using a polarimeter.

| | **Angle of rotation, $OC^{O}$** | | | | | |
|---|---|---|---|---|---|---|
| Concentration | Fructose standard | Glucose standard | Sucrose standard | Maltose standard | Lactose standard | Standard mixture |
| 2% | -3.1 | +1.0 | +1.2 | +1.1 | +1.1 | +0.7 |
| 4% | -11.7 | +2.8 | +4.0 | +3.3 | +2.5 | +1.1 |
| 6% | -16.7 | +5.3 | +6.8 | +5.8 | +4.1 | +3.2 |
| 8% | -19.6 | +7.9 | +9.8 | +6.2 | +5.2 | +4.8 |
| 10% | -22.6 | +9.5 | +13.2 | +8.2 | +6.2 | +5.2 |
| 15% | -24.9 | +11.7 | +16.7 | +10.2 | +9.7 | +7.7 |
| 20% | -30.2 | +14.7 | +18.2 | +13.7 | +11.2 | +8.7 |

Unknown (10%): Angle of rotation, $\alpha^{O} = +3.5$, temperature = $29^{O}C$

Fig. I the plot of various sugar standards and standard mixture using polarimeter.

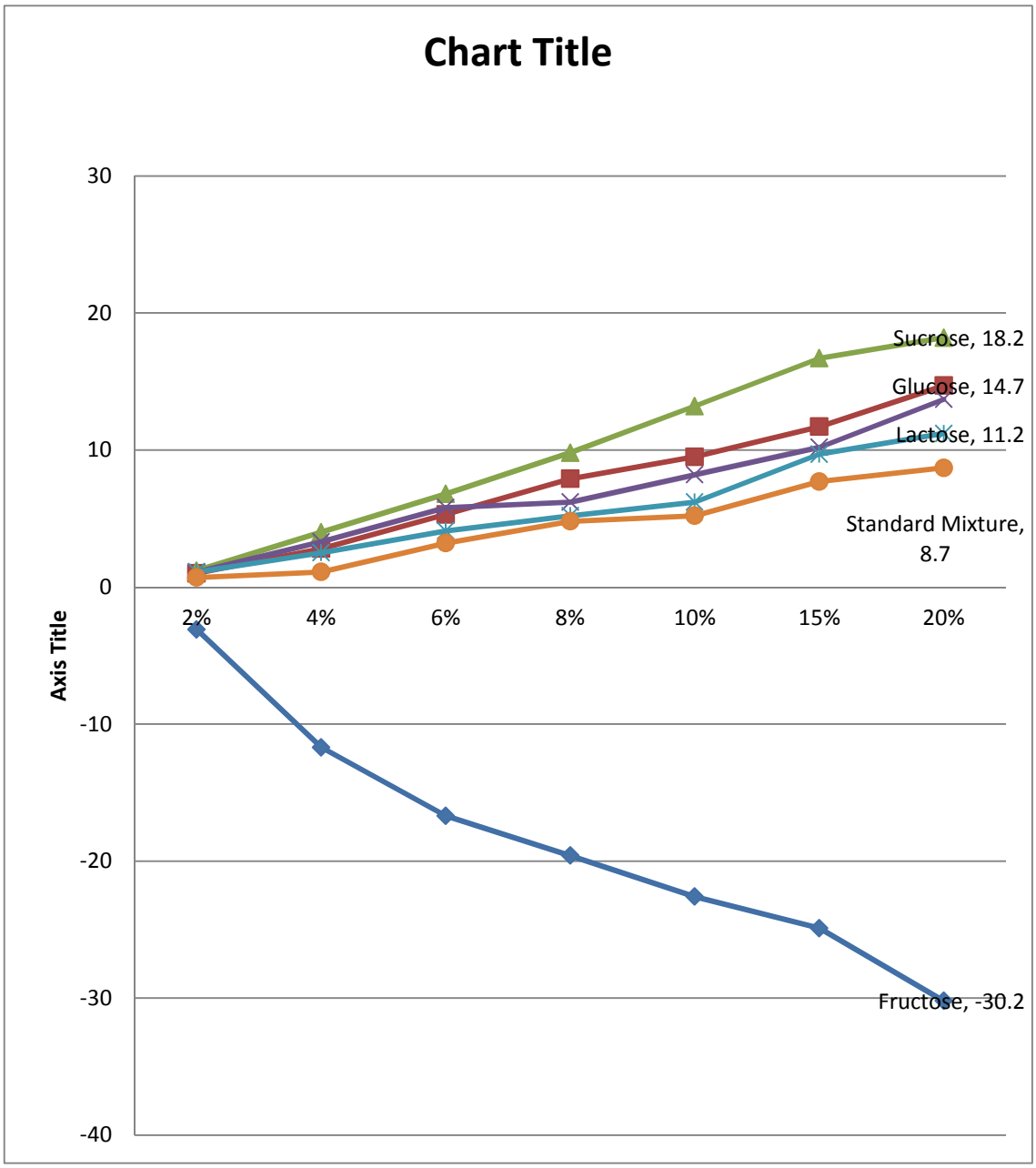


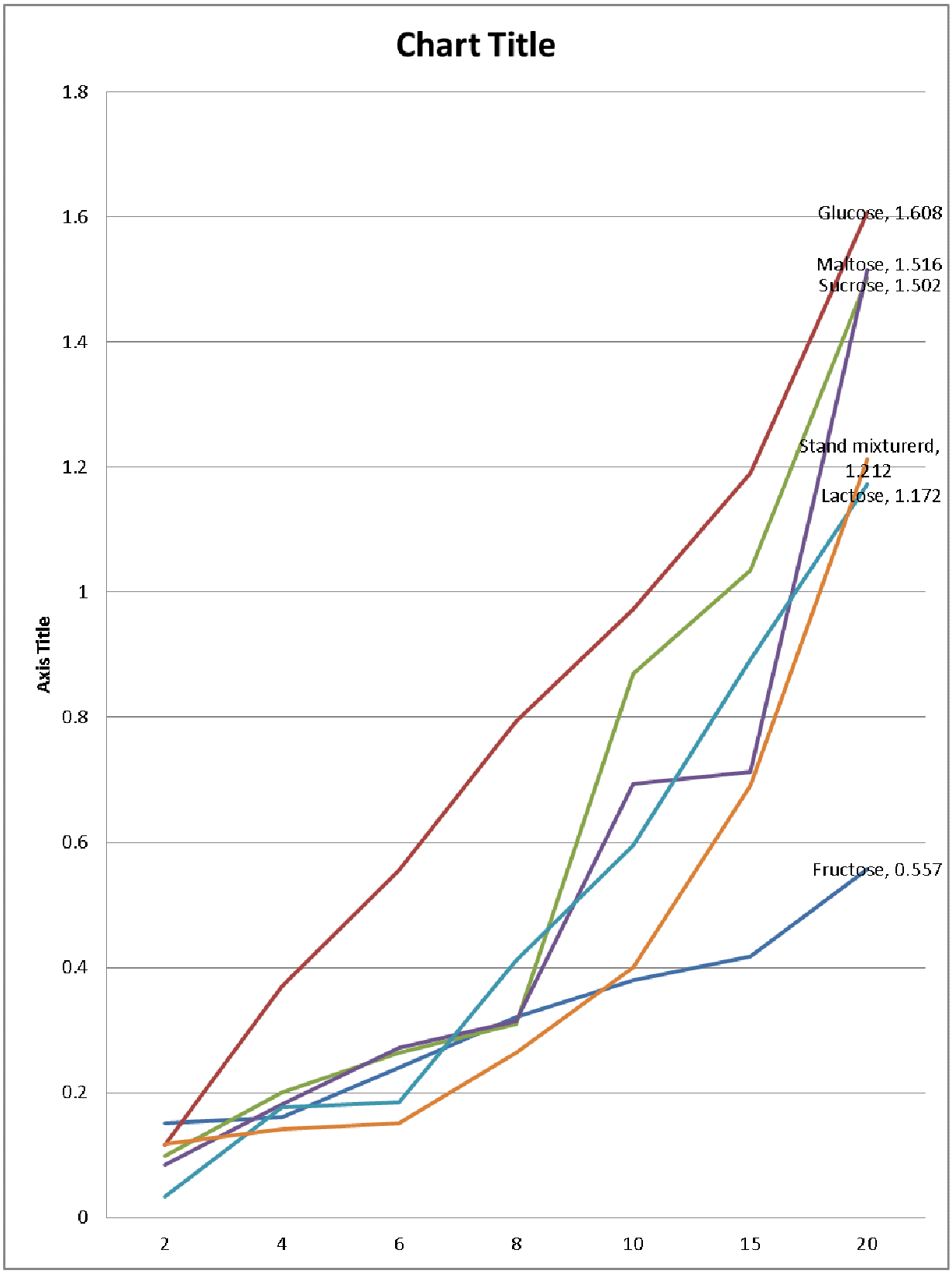

Table 2: show the readings obtained with various sugar standards and standard mixture using µv-visible spectrophotometic

| Concentration | Absorbance at 490nm | | | | | |
|---|---|---|---|---|---|---|
| | Fructose standard | Glucose standard | Sucrose standard | Maltose standard | Lactose standard | Standard mixture |
| 2% | 0.151 | 0.116 | 0.099 | 0.085 | 0.034 | 0.117 |
| 4% | 0.160 | 0.370 | 0.200 | 0.181 | 0.176 | 0.142 |
| 6% | 0.240 | 0.555 | 0.263 | 0.271 | 0.185 | 0.151 |
| 8% | 0.321 | 0.794 | 0.310 | 0.314 | 0.412 | 0.263 |
| 10% | 0.38 | 0.973 | 0.869 | 0.693 | 0.595 | 0.400 |
| 15% | 0418 | 1.191 | 1.035 | 0.713 | 0.892 | 0.690 |
| 20% | 0.557 | 1.608 | 1502 | 1.516 | 1.72 | 1.212 |

Unknown (10%): Absorbance at 490nm = 0,.562
Phenol – sulphuric acid method

Dilution factor in each case = x200
Fig. 2: Determination of various sugars and their standard mixture using µv-visible spectrophotometry.




ABS = 0.0227 Fructose (%) 0.1077 $R^2$ = 0.9582
Abs = 0.0817 Glucose (%) +0.0508 $R^2$ = 0.9778

Abs = 0.0811 Sucrose (%) +0.422 $R^2$ = 0.9442
Abs = 0.0653 Lactose (%) +0.1112 $R^2$ = 0.9866
Abs = 0.0614 standard mixture – 0.1364 $R^2$ = 0.9412

The results presented in table 1 and 2 shows that the results obtained using polarimeter and µv-visible spectrophotometer with the various sugar standard and standard mixture. Figures 1 and 2 show the plots of various sugars with concentration of polarmetery and µv-visible spectrophotemenly. The results show that µv-visible is more sensitive than polarimetryUv-visible spectrophotometric method was most sensitive for lactose ($R^2$ = 0.99) and glucose ($R^2$ = 0.98) and is less sensitive to maltose ($R^2$ = 0.91).

Table 3 ANOVA: Two factor without Replication Uv – Visible Spectrophotometry

| Summary | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 2 | 6 | 0.602 | 0.100333 | 0.001545 |
| 4 | 6 | 1.23 | 0.205 | 0.006914 |
| 6 | 6 | 1.665 | 0.2775 | 0.020645 |
| 8 | 6 | 2.414 | 0.402333 | 0.039171 |
| 10 | 6 | 3.97 | 0.661667 | 0.053069 |
| 15 | 6 | 4.939 | 0.823167 | 0.075789 |
| 20 | 6 | 7.627 | 1.271167 | 0.158815 |
| | | | | |
| Fructose | 7 | 2.228 | 0.318286 | 0.021599 |
| Glucose | 7 | 5.667 | 0.809571 | 0.274735 |
| Sucrose | 7 | 4.278 | 0.611143 | 0.280498 |
| Maltose | 7 | 3.773 | 0.539 | 0.244208 |
| Lactose | 7 | 3.466 | 0.495143 | 0.173868 |
| Standard Mixture | 7 | 3.035 | 0.433571 | 0.161096 |

**ANOVA**

| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 6.136593 | 6 | 1.022766 | 38.38061 | 9.11E-13 | 2.420523 |
| Columns | 0.9803 | 5 | 0.19606 | 7.357405 | 0.000133 | 2.533555 |
| Error | 0.799439 | 30 | 0.026648 | | | |
| Total | 7.916332 | 41 | | | | |

Table 4: Shows Anova: two factor without replication polarimetry

| Summary | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 2 | 6 | 8.2 | 1.366667 | 0.750667 |
| 4 | 6 | 25.4 | 4.233333 | 14.31067 |
| 6 | 6 | 41.9 | 6.983333 | 24.26167 |
| 8 | 6 | 53.5 | 8.916667 | 30.81767 |
| | 6 | | | |
| | 6 | 64.9 | 10.81667 | 41.19367 |
| 10 | 6 | 80.9 | 13.48333 | 40.48167 |
| 15 | 6 | | | |
| 20 | 6 | 96.7 | 16.11667 | 57.9416 |
| | | | | |
| Fructose | 7 | 128.8 | 18.4 | 80.40667 |
| Glucose | 7 | 52.9 | 7.557143 | 23.79952 |
| Sucrose | 7 | 69.9 | 9.985714 | 41.01476 |
| Maltose | 7 | 48.5 | 6.928571 | 17.85238 |
| Lactose | 7 | 40 | 5714286 | 13.45143 |
| Standard Mixture | 7 | 31.4 | 4.485714 | 9.358095 |

**ANOVA**

| *Source of Variation* | *SS* | *Df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 953.6557 | 6 | 158.9426 | 29.49911 | 2.66E-11 | 2.420523 |
| Columns | 887.1469 | 5 | 177.4294 | 32.93018 | 2.56e-11 | 2.533555 |
| Error | 161.6414 | 30 | 5.388048 | | | |
| Total | 2002.444 | 41 | | | | |

Polarimetry is less sensitive for fructose ($R^2$ = 0.86) as can been seen in fig. 1. The results also suggest that standard mixture cannot be used effectively for fructose ($R^2$ = 0.86).
The difficult with polarmetric determination of fructose may be due to the fact that while other sugars are dextrorotatory with plus (+) sign, fructose is laevorotatory with minus (-) sign.
Fructose is also a ketohexose or alldohexose containing sugar which the others are either aldohexose or alldohexose containing sugars. These characteristics of fructose could affect the magnitude and direction of rotation. The presence of fructose in sucrose could be similarly account for sucrose being next in terms to low sensitivity in polarimetry
Figure 2 shows the determination of various sugars and their standard mixture using µv-visible spectrophotometry. The line for fructose using µv-visible spectrophotometry is quite off from the other lines. The reason maybe attributed to the fact that the sensitivity of chemical reagents for this method is not equally specific for both glucose and fructose unit. Since most of the other sugars are either glucose or glucose containing units.
Table 3 and 4 shows the analysis of variance for each of the two different methods, polarimetry and µv-visible spectrophotometer respectively. This result shows that there is significant difference ($P \leq 0.05$) at different concentrations and that there is also significant difference ($P \leq 0.05$) among sugars for each of the method. There is a significant difference ($P \leq 0.05$) among sugars and that also significant difference in the two methods from the results obtained.

## CONCLUSION

Many common methods are used by researcher,some researchers just made a choice merely out of interest without any statistical evidence to back their choice. Works of similar evaluations show that sensitivity, limit of detection, reliability and validity of methods differences. Thereis a need to determine a discrimination procedure for evaluating monosaccharaides and disaccharides using polarimetry and µv-visible spectophometry.
The results presented have shown that µv-visible spectrophometry can be used to determine the sugars, glucose, sucrose, maltose, lactose and their standard mixture with the highest sensitivity of about ($R^2$ = 0.99) being better than the polarimetric method. This implies that monosaccharaides and good number of disaccharides can be determined with µv-spectrophotometry with acceptable or top degree sensitivity.
Polarimetry is less sensitive method ($R^2$ = 0.94) to µv-visible septrophotometry in sugar determination. But it was able to identify higher percentage of specific monosaccharaides. This was proved by the significant different among sugars for each method. Polarimatry and µv-visible spectrophotometry did not show good sensitivity for the determination of fructose.
Fructose showed remarkables variation in sensitivity ($R^2$ = 0.86), for the polarimetric method,this could be due to the different in its direction of rotation as well as its magnitude. Fructose has different response to chemical reagent used in sugar determination as opposed to all the other sugars which are either glucose containing units. This might have accounted for the line for fructose in figure 2 being quite off from the lines of the other sugars in the µv-visible spectrophotometry determination.
UV-visiblespectrophometry are more sensitive instrument to use in determination of monosaccharide and disaccharide than polarimetry.

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