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Research Article

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Determination of Sugar in Cane Juices using High Performance Liquid Chromatography HPLC and Ultra violet UV at Kenana Sugar Factory – Sudan

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ABSTRACT

This Study was done in Kenana Sugar Factory in the Republic of Sudan. In this study sugar content determined using High Performance Liquid Chromatography HPLC and Ultra violet UV. The samples of various sugar cane juices and bagasse was taken during the industrial processes. The losses of sugar in bagasse also determined using those methods. The results of this experiment were shown in tables and graphics. The main reasons of the high losses in Kenana factory had been determined as one of the results of this study

Keywords*:* Sugar losses; HPLC; Kenana sugar company; Chromatography; Ultra violet; Determination

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INTRODUCTION

Cane juice contained sucrose and little amounts of glucose and fructose, in a certain proportions. Any increase of the quantity of glucose and fructose refer to sucrose inversion, so it must be rottenly determined these sugars during the industrial processes in sugar factories to control the sugar quality and losses.
The common apparatus which is widely used in sugar factories in determining sugars is the polarimeter, it exploits the fact that certain crystals and molecules are able to polarized beams of light.
Polarimeter (sometimes called a polari-scope) is used to measure the degree of rotation. In the course of time scientists in the sugar industry found empirically a convenient range of concentrations and tube lengths for examining sucrose solutions, and took matters a step further by calibrating their polarimeters not in angular degrees but directly and more conveniently in sugar degrees that is in per cent sucrose. These specialized, instruments are called saccharimeters. Thus if a standard or 'normal' weight (26 g) of pure sucrose ,is dissolved in a 'standard' volume of distilled water (100 ml) and examined in the 'standard length of tube (200 mm) the saccharimeter will indicate 100% sucrose, or 100° polarization (pol) when the analyzer has been turned to compensate for the rotation of the light beam caused by the solution, If the sample is impure then a lower reading will be given, reflecting precisely the proportion of impurity present.
The impurities present in a sugar solution might themselves be optically active and so exert an influence, on the net polarization of the material under examination. Two of the most important of these are glucose (dextrorotatory) and fructose (laevorotatory), besides occurring naturally in sugar-cane juice, they also are the products of the hydrolysis of sucrose. Since an equimolecular solution of these two compounds is laevorotatory, collectively they are called invert sugar (because they change the direction of net polarization). The greater the proportion of optically active compounds in the sample the less I precise is the measurement of sucrose by polarization.

MATERIALS AND METHODS

Materials

In order to find out the concentration of the pure sucrose, the concentration of the invert sugars (glucose and fructose) must be known. It is easy to determine the concentration of these sugars since they are reduced Fehling

solutions into cubber (Cu+), so they are called reduced sugars (RS). The percentage of pure sucrose (ratio%) calculated as followed:

$$Ratio = \frac{reduced\ sugars, \%}{sugars, \%} = \frac{RS}{Pol}, \%$$

High performance liquid chromatography (HPLC) is a form of column chromatography used frequently in biochemistry and analytical chemistry. It is sometimes referred to as high pressure liquid chromatography. HPLC is used to separate components of a mixture by using a variety of chemical interactions between the substance being analyzed and the chromatography column. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences (fig. 1) in their partitioning behavior between the mobile liquid phase and the stationary phase. HPLC is a popular method of analysis because it is accuracy, easy to learn and use and is not limited by the volatility or stability of the sample compound.

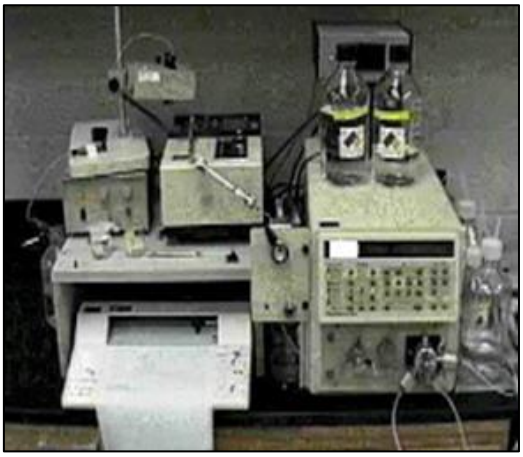

Figure 1: HPLC

A spectrophotometer measures the absorbance of light as a function of energy (or wavelength). There are several different designs commercially available. A simple schematic of this type of device is shown.
Light of wavelengths corresponding to the Ultra violet and a visible wavelength (the source is a deuterium/Tungsten lamp) passes through a sample in a cuvette, where some of the light is absorbed in electronic transitions in the analyzer. The transmitted light is passed through a slit and dispersed along a diode array, where the amount of light of different wavelengths can be determined. This is compared to a previously measured blank, to determine wavelengths at which the sample undergoes electronic transitions (figure 2).

Methods

To determinate the sugars in cane juice using HPLC, 1 ml of the cane juice was diluted to 100 ml with distilled water, 0.1 ml from the diluted cane juice was injected into HPLC, the direct reading of sugars concentration were determined. The result of this experiment shown in Table 1;

For the determination of sucrose in cane juice using UV, 2.5 ml of the cane juice was diluted to 100 ml with distilled water, a little amount from the diluted juice was taken in the UV cell and the absorption of sugar was determined. To determine the concentration a different weighs of pure sucrose was taken and dissolved in distilled water in a 100ml volumetric flasks and diluted to the volumetric marks with distilled water and their absorption was determined in UV. The result of this experiment shown in table 2.

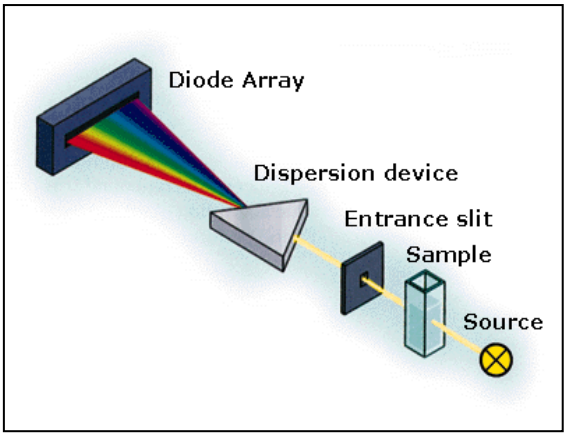

Figure 2: Electronic transitions

For the determination of sugar content in bagasse (pol) using polarimeter, 500ml distilled water and 5ml Na_2CO_3 were added to 100gm bagasse sample in a weighed beaker, and was boiled in a water bath for one hour, then cooled to about 95^oC, weighed. Juice was extracted from the mixture by pressing, and was cooled to a room temperature. The extracted juice was treated with dry $Pb(Ac)_2$, filtered and polarized. The result of this experiment shown in table 3.

For the determination of sugar content in cane juices (pol) using polarimeter, a minimum quantity of dry lead acetate was added to about 80-90 ml of the juice samples in a 100ml flask, mixed well, and filtered. The filtrate was trans-ferred to 200mm calibrated pol tubes and polarized. The result of this experiment shown in table 4.

To determinate the sugar content in mud (Pol) using polarimeter, 25g from the mud sample was weighed and dissolved in distilled water in a 200ml volumetric flask. 4ml from the Pb(AC)2 solution was added and diluted to the volumetric mark with distilled water, the mixture was filtered and the filtrate was transferred to 200mm calibrated pol tube and polarized. The result of this experiment shown in table 5.

The determination of solid content (brix) in cane juices was done using the Refractometer. The (zero) point of the Refractometer scale was checked with distilled water. A drop of the juice sample was added over a prism surface and the folder cover was closed over the prism surface. The intersecting line gave the direct reading of the total dissolved solid content in the given sugar so-lutions. The result of this experiment shown in table 6.

Determination of the pH of cane juices done using the pH meter. The pH meter was checked with standard buffer solutions, then the pH of the samples of solutions were determined. The result of this experiment shown in table 7.

The determination of reduced Sugar in juices (RS) done using the titration method. For this, 25g of the filtrate juice sample was weighed in a 200ml volumetric flask, and diluted to the volumetric mark with distilled water. The solution was filtered, and the filtrate was titrated with a mixture of 5ml Fehling A and 5ml of Fehling B using methyl blue as an indicator. The titration was continued until the Fehling's color changed into pink color. The result of this experiment shown in table 8.

RESULTS AND DISCUSSION

Injecting pure soluble of different concentrations of sucrose, fructose and glucose in HPLC instrument, the retention time of each sugar will be detected and the absorbance of each concentration is translated in chromatogram as shown in figure (1).
All this information had been stored in the computer system attached with the used HPLC instrument using specific program. The computer automatically detects these sugars at all samples and calculates their concentration. So when injecting the examined cane juice sample in HPLC, the direct result was sent as shown in table (1).

Table 1: Sugars content in cane juice using HPLC

Substance	Sucrose	Fructose	Glucose
HPLC reading	13.58	1.2	0.81

Table 2: sucrose concentration In cane juice using UV

Sucrose concen-tration%	0.2	0.7	1.2	2.2	3.2	5.2	8.2
Absor-bance at 210nm	0.006	0.007	0.02	0.32	0.052	0.09	0.16

The relation between the concentration of sucrose and the absorbance had been modeled as shown in figure (3) by the equation $y = 0.0184x - 0.0042$

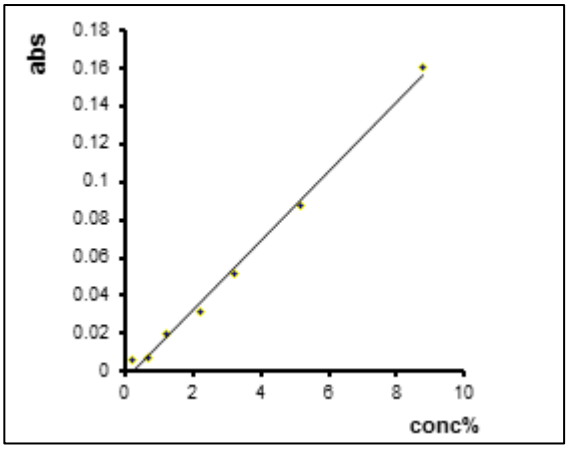

Figure 3: Influence of sucrose concentration on absorption reading

Mixed juice sample absorbance reading: 0.244
Sucrose concentration according to the relation $y = 0.0184x - 0.0042$ is 13.5
The result of determination of sugar content using polarimeter in bagasse shown in table (3).

Table 3: Sugar content (pol%) in bagasse

Time, hrs	1	2	3	4	5	6
Bagasse Pol%	2.12	2.13	2.3	2.42	2.78	3.3

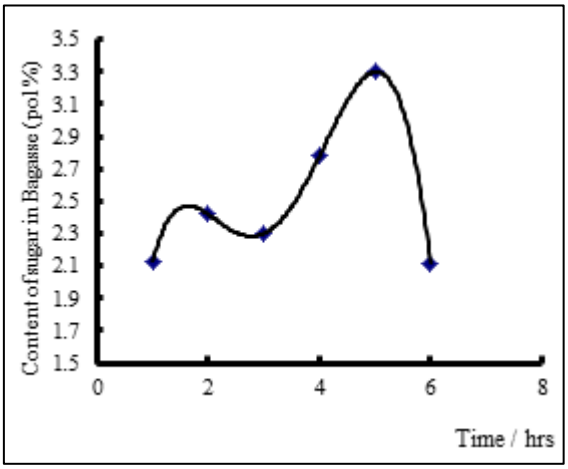

Figure 4: Sugar content (pol%) in bagasse

The result of determination of sugar content using polarimeter in cane juices shown in table (4);

Table 4: Sugar content (pol%) in cane juices

juice / hr	5	4	3	2	1
Mixed juice	12.21	12.97	11.99	13.56	14.48
Clear juice	13.44	13.73	14.26	14.48	14.94
Syrup	52	51.52	52.04	56.44	56.26
Final molasse	30.8	29.48	30.36	30.8	28.6

The result of determination of pH in cane juices is shown in table (6)

Table 6: pH in samples of cane juices

juice / hr	5	4	3	2	1
Mixed juice	5.3	5.2	5.5	5.3	5.2
Clear juice	6.78	6.61	6.98	6.78	6.79
Syrup	6.17	6.28	6.2	5.81	5.78

Analyzing the influence of pH and temperature on sucrose decomposition (Ratio)
Table 7 – The rate of sucrose decomposition (ratio%) in various pH & temperatures (summarization of table (7).
The result of determination of solid content (brix%) in cane juices is shown in table 5.

Table 5: Solid content (brix%) in samples of cane juices

Juice / hr	5	4	3	2	1
Mixed juice	14.73	15.07	14.28	15.33	16.96
Clear juice	15.93	15.82	17.06	16.85	17.25
Syrup	61.64	61.59	61.75	71.46	64.63
Final molasse	87.68	81.22	88.68	86.64	80.16

Determination of sucrose in cane juice using HPLC:
From the result presented in table (1) it can be clear that HPLC is more accurate in reading sucrose and other sugars, furthermore it gives a direct fast reading because of it is computerize feeding system.

Determination of sucrose in cane juice using UV:
It is possible to calculate the sucrose concentration using UVvis after determining the wave length of sucrose and relate between multi sucrose concentrations and their absorptions (table (1) figure (1)).

RECOMMENDATIONS

It is possible to minimize the amount of sugar that lost due to chemical reasons by the following:
* The pH of the cane juice at the different operations should range between (7-8) (this range is according to the temperature degree), to protect the sucrose from the reaction of decomposition.
* It is necessary to add some lime to mud to bring the pH to 7.8-8.0 and to maintain the mud temperature at 85oC after bagacillo addition.

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