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# COMPARISON OF HPLC AND TRADITIONAL ANALYTICAL METHODS FOR THE ESTIMATION OF SUGARS IN MOLASSES

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## ABSTRACT

Samples of cane and beet molasses were obtained from Armant sugar factory and Delta company of sugar, Qena and Kafr El-Sheikh governorates; respectively, during 2002/2003 working season. Sucrose and reducing sugars were determined by HPLC and classical analytical methods.

Results revealed that, the sucrose determined by HPLC technique was from 3.30 % to 10.26 % lower than that determined by a double polarization in beet and cane molasses; respectively. This was due to generation more compounds after acid treatment have dextrorotation of light. In the same trend, reducing sugars determined by HPLC was lower than that of a traditional methods ( Ofner's and Fehling methods in beet and cane molasses , 0.21 % to 7.41 %; respectively ) because of many organic and inorganic substances in malasses, that can reduce copper as well as glucose and fructose. The glucose / fructose ( G / F ) ratios using HPLC were ranged from 0.68 : 1 in cane molasses to 1.18 : 1 in beet molasses compared to traditional methods, i.e. 1 : 1 mixture of glucose and fructose. The average value was 0.77 : 1 in cane molasses compared to 0.93 : 1 in beet molasses.

The HPLC determination of sugars in molasses was a rapid , accurate and repeatable to emphasis the results of analysis. It is also very important for calculation sugar loss in final molasses and exhaustion of molasses.

## INTRODUCTION

Sugar constitutes approximately three-quarters of the total solids content of molasses. Sucrose accounts approximately half of the sugar content of molasses, while reducing sugars consists of approximately equal quantities of glucose and fructose account for the remainder of the sugar content. The non-sugar constituents of molasses include approximately 10 percent ash, 5 percent nitrogenous organic matter and 10 percent miscellaneous materials which include organic matter, gums, acids and other materials ( FAO, 2001 ).

Final molasses is the heavy viscous mother liquor remaining after crystallization of sugar from which is not possible to extract more sucrose by convential procedures, it is an excellent raw material for fermentation owing to its high sugar content and probiotic properties, which promote microbial growth ( Otero *et al.*, 1993 ). Final beet molasses has an unpleasant taste and is not normally used for human food. On constract, final cane molasses does have some food use, normally in the form of treacle which is clarified molasses ( Edwards, 2000 ).

The determination of absolute and relative quantities of sucrose, glucose and fructose in molasses has been an important problem to the

sugar industry for many years. The sugar in molasses have been lost to sugar production; the greater the quantity of sugar in final molasses, the lower the yield of the factory ( Clark and Brannan, 1978[a] ). Molasses contains monosaccharides ( glucose and fructose ), disaccharides ( sucrose ), some trisaccharides and oligosaccharides; because of the complex composition of molasses, the analysis of the sugars is so difficult. Traditional optical methods work poorly, because of presence many optically active compounds in the sample. Similarly, oxidation and reduction methods lack accuracy because there are many other reducing compounds presents as well as glucose and fructose. In comparison of analysis of sugar in molasses by isotop dilution, gas liquid chromatography, chemical methods and polarization; it was calculated that the pol data gave no useful information ( Kort *et al.*, 1975 ).

For this reasons, this work was done to determine true sucrose in molasses by HPLC, and comparison with optical methods; as well as determination of glucose and fructose and their ratio ( G / F ) by HPLC which comparison with traditional methods (Ofner's and Fehling volumetric methods for beet and cane molasses; respectively ).

## MATERIALS AND METHODS

**Materials:**

The molasses samples, i.e. cane and beet molasses were collected from Armant sugar factory and Delta company of sugar, Qena and Kafr El-Sheikh governorates; respectively during 2002/2003 working season.

A sample of approximately 20 gm of molasses was weighted out and diluted to approximately 200 gm with deionized water. Although only a few milligrams were actually required for HPLC analysis, a large sample was taken for the dilution to minimize error in sampling.

Methods

**Chemical analysis:**

Sucrose content ( double polarization ) was determined according to De Whalley ( 1964 ) method.

Reducing sugars was determined by Lane-Eynon volumetric method as described by Payne ( 1968 ) for cane molasses, and determined by Ofner's volumetric method as described by De Whalley ( 1964 ) method for beet molasses.

The HPLC system was equipped with a Bio-Rad Aminex HPX-87K cation exchange column 300 × 7.8 mm Kept at 85°C and a waters 410 RI detector, thermostatted intermally at 45°C. The mobile phase ( 0.01 M $K_2SO_4$ ) was pumped at 0.5 ml / min and 20 µl of the diluted sample was injected with a Bio-Rad refrigrated AS-100 autoinjector, the sample was diluted as 4 g / 100 ml volume with de-ionized water to give higher concentrations in the collected fractions. By standard solutions and retention times, sugars can be identified ( Michael and Jue, 1994 ).

**Statistical analysis:**

The data were statically analysed according to SAS ( 1990 ) using SAS computing procedure.

## RESULTS AND DISCUSSION

The percentages of sucrose and reducing sugars in the series cane and beet molasses samples by the different methods are shown in Tables 1, 2, 3 and 4 as well as in Fig ( 1 ). In cane molasses, sucrose was determined by double polarization ( clearget method ); with acid invertion ( De Whalley, 1964 ), and reducing sugars by a Lane - Eynon titration. In beet molasses, sucrose was determined by a double polarization, and reducing sugars by Ofner's volumetric method ( De Whalley, 1964 ). The difference in reducing sugars measurement comes primarily from the high content in sugar cane molasses of colloids and other suspended matter, high content of divalent ions, high colour and high content of invert sugars than beet molasses ( Saska and Lancrenon, 1994 ).

The results presented in Tables 1 and 3 indicate that in both beet and cane molasses samples contain a considerable compounds other than sucrose that cause dextrorotation of light after acid treatments. Therefore, the sucrose determined by HPLC is from 3.30 % ( beet molasses) to 10.26 % (cane molasses) lower than the sucrose determined by a clearget methods with acid inversion.Also, reducing sugars measurments by HPLC was lower than that of a traditional methods ( Ofner's and Fehling volumetric methods in beet and cane molasses, i.e. 0.21 % to 7.41 %; respectively ) as shown in Tables 2 and 4..

In the sucrose determination by a clearget method, additional errors arise from the acid hydrolysis of trisaccharides and higher molecular weight saccharides with break down to components that affect the optical rotation, apparently in a positive manner like glucose ( Clarke and Brannan, 1978[b] ). The matter of G / F ratio ( Tables 2, 4 and Fig 2 ) comes into this calculation. In the traditional methods, the calculation is based on a 1 : 1 mixture of glucose and fructose, whereas the ratios in actuality ranged from 0.68 : 1 in cane molasses as shown in Table 2 to 1.18 : 1 in beet molasses ( Table 4 ) by HPLC technique. Since, the G / F ratio is less than 1 in most cases, there is a consistent lowering of the initial polarization. The correlation coefficient for sucrose by HPLC with sucrose by a cleaget method of cane molasses, $r$ = +0.722; and $r$ = +0.935; so close to 1 for reducing sugars by HPLC technique with it using Fehling volumetric method, indicates that there is a fairly constant error in the sucrose pol measurment. In Table 4 ( beet molasses ), in two samples, 2 and 4; the G / F ratio is greater than 1, and the sucrose ( HPLC technique ) values for these two samples as shown in Table ( 3 ) are closer to the results for sucrose by the clearget method. For sample 2, G / F ratio = 1.03; difference in sucrose analysis = 3.42, and for sample 4, G / F ratio = 1.18; difference in sucrose analysis = 3.30, compared to the average differences over the other eleven samples of 7.71 for beet and

cane molasses samples. There is no excess fructose in their samples to cause an increase in the negative error of the initial polarization reading.

The results from glucose and fructose measurements by HPLC compared to those from the reducing sugars titration emphasize the unsuitability of the latter for invert sugar analysis in molasses, the low correlation coefficient; r = +0.464 for glucose with fructose of beet molasses using HPLC technique corroborate this ( Table 4 ). There are many organic and inorganic substances in molasses that can reduce copper as well as glucose and fructose. Apparently, the large inconsistencies were not present with Fehling and Ofner's volumetric methods.

Kolekar and Keskar ( 1998[a] ) estimated that reducing sugars in cane molasses by traditional analysis and HPLC technique was 11.45 and 10.62 %; respectively.

Kolekar and Keskar ( 1998[b] ) stated that during crystallization, the reducing sugars tend to react with non-sugars ( e. g. amino acids ) to undergo Millard reactions and form various brown products. Monosaccharides were initially formed in equal proportion, but glucose was subsequently found at slightly higher concentrations than fructose. Interestingly, the proportion of ketoses also increased, hence it is not unreasonable to assume that some of fructose was utilised in the formation of the trisaccharides.

The fact that the true sucrose in molasses is rather lower than the double polarization value emphasizes the importance of exhaustion of molasses and could affect calculation of loss in molasses, and high concentration of salts increase the sucrose solubility ( via molassigenic effects ) resulting in high final molasses, thus cane molasses contain more sucrose than beet molasses ( Aftab, 1998 ).

The HPLC determination of sugars in molasses is a rapid, accurate and repeatable to emphasis results of analysis. Consequently, the HPLC technique is recommended to be used in our sugar factories as supported by Kolekar and Keskar, 1998[a] in their research.

Conclusion

The results revealed that, HPLC technique was much better than that of traditional methods on determination of true sugars, because of their superior in rapidity, accuracy and repetation to emphasis results. Thus, it will be very economical to change the traditional methods on sugar determination by HPLC technique in our factories for calculation molasses exhaustion and sugar loss in final molasses, whereas losses through final molasses have always been a matter of great concern for the sugar industry technologists.

Table ( 1 ): Sucrose concentration in cane molasses.

| Sample | Sucrose | | Difference | Total Sugars | | Difference |
|---|---|---|---|---|---|---|
| | HPLC[1] | Clearget[2] | | HPLC | Classical | |
| 1 | 29.18 | 38.32 | - 9.14 | 43.08 | 59.54 | - 16.46 |
| 2 | 29.49 | 37.82 | - 8.33 | 40.83 | 54.97 | - 14.14 |
| 3 | 29.00 | 39.26 | - 10.26 | 37.75 | 55.42 | - 17.67 |
| 4 | 30.18 | 37.62 | - 7.44 | 39.61 | 53.87 | - 14.26 |
| 5 | 31.02 | 39.54 | - 8.52 | 40.84 | 54.29 | - 13.45 |
| 6 | 31.21 | 39.14 | - 7.93 | 39.75 | 53.56 | - 13.81 |
| 7 | 30.30 | 38.76 | - 8.46 | 37.65 | 52.38 | - 14.73 |
| 8 | 32.91 | 40.62 | - 7.71 | 43.21 | 56.83 | - 13.62 |
| Average | 30.41 | 38.88 | - 8.47 | 40.34 | 55.11 | - 14.77 |

$r^{(1,2)} = + 0.722$

Table (2): Concentrations of glucose and fructose in cane molasses.

| Sample | Glucose[3] | Fructose[4] | Total R.S. (HPLC)[1] | Total R. S. (Lane & Eynon)[2] | Difference | G / F ratio |
|---|---|---|---|---|---|---|
| | HPLC | | | | | |
| | 6.06 | 7.84 | 13.90 | 21.22 | - 7.32 | 0.71 |
| 2 | 4.77 | 6.62 | 11.39 | 17.15 | - 5.76 | 0.73 |
| 3 | 3.82 | 4.93 | 8.75 | 16.16 | - 7.41 | 0.77 |
| 4 | 4.30 | 5.13 | 9.43 | 16.25 | - 6.82 | 0.84 |
| 5 | 4.39 | 5.43 | 9.82 | 14.75 | - 4.93 | 0.81 |
| 6 | 3.75 | 4.79 | 8.54 | 14.42 | - 5.88 | 0.78 |
| 7 | 3.36 | 3.99 | 7.35 | 13.62 | - 6.27 | 0.90 |
| 8 | 4.17 | 6.13 | 10.30 | 16.21 | - 5.91 | 0.68 |
| Average | 4.33 | 5.61 | 9.94 | 16.22 | - 6.28 | 0.77 |

$r^{(1,2)} = + 0.935$
$r^{(3,4)} = + 0.948$

Table ( 3 ): Sucrose concentration in beet molasses.

| Sample | Sucrose | | Difference | Total Sugars | | Difference |
|---|---|---|---|---|---|---|
| | HPLC[1] | Classical[2] | | HPLC | Classical | |
| 1 | 44.27 | 50.41 | - 6.14 | 45.14 | 51.56 | - 6.42 |
| 2 | 47.64 | 51.06 | - 3.42 | 48.27 | 52.05 | - 3.78 |
| 3 | 45.69 | 51.58 | - 5.89 | 46.59 | 52.70 | - 6.11 |
| 4 | 47.33 | 50.63 | - 3.30 | 48.31 | 51.92 | - 3.61 |
| 5 | 46.41 | 51.38 | - 4.97 | 47.11 | 52.29 | - 5.18 |
| Average | 46.27 | 51.01 | - 4.74 | 47.08 | 52.10 | - 5.02 |

$r^{(1,2)} = + 0.217$

Table ( 4 ): Concentrations of glucose and fructose in beet molasses.

| Sample | Glucose(3) | Fructose(4) | Total R. S. (HPLC) (1) | Total R. S. (Ofner's) (2) | Difference | G / F ratio |
|---|---|---|---|---|---|---|
| | HPLC | | | | | |
| 1 | 0.38 | 0.49 | 0.87 | 1.15 | - 0.28 | 0.78 |
| 2 | 0.32 | 0.31 | 0.63 | 0.99 | - 0.36 | 1.03 |
| 3 | 0.39 | 0.51 | 0.90 | 1.12 | - 0.22 | 0.76 |
| 4 | 0.53 | 0.45 | 0.98 | 1.29 | - 0.31 | 1.18 |
| 5 | 0.33 | 0.37 | 0 70 | 0.91 | - 0.21 | 0.89 |
| Average | 0.39 | 0.42 | 0.81 | 1.09 | - 0.28 | 0.93 |

$r^{(1,2)} = + 0.909$
$r^{(3,4)} = + 0.464$

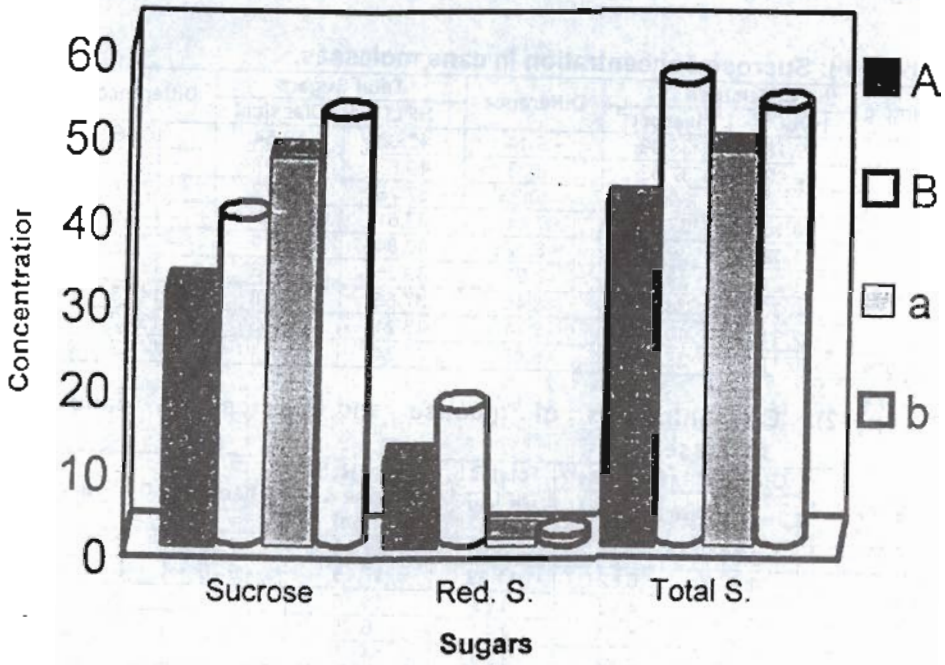


Fig. (1): Average values of sucrose, reducing sugars and total sugars of cane and beet molasses by HPLC and traditional methods.
A = HPLC technique of cane molasses.
a = HPLC technique of beet molasses.
B =Traditional methods of cane molasses.
b = Trditional methods of beet molasses.

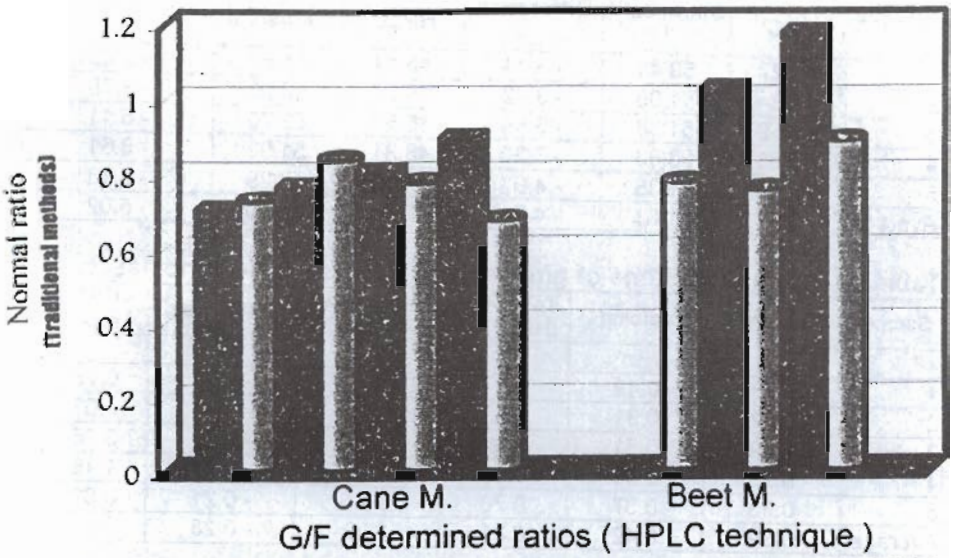


Fig. ( 2 ): G / F ratios in the series cane and beet molasses by HPLC technique.

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# مقارنة طريقة HPLC والطرق التقليدية لتقدير السكريات في المولاس

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تم الحصول على عينات من مولاس القصب ومولاس البنجر من مصانع السكر بأرمنت – محافظة قنا ، وشركة الدلتا للسكر – محافظة كفر الشيخ خلال موسم التصنيع ٢٠٠٢ / ٢٠٠٣ على التوالي ، وتم تقدير السكروز والسكريات المختزلة بطريقة HPLCوطرق التقدير التقليدية.

أشارت النتائج بأن السكروز المقدر بطريقة HPLC أقل بمعدل ٣,٣٠ % – ١٠,٢٦ % من السكروز المقدر بطريقة الاستقطاب بعد التحول وذلك لمولاس البنجر والقصب على التوالي ، ويعزى ذلك الى أن المعاملة بالحامض في طريقة الاستقطاب تؤدي الى تخليق مركبات أخرى جديدة لها نشاط ضوئي.

لوحظ أيضا أن السكريات المختزلة المقدرة بطريقة HPLC أقل عن نظيرتها المقدرة بالطرق التقليدية ( طريقة اوفنر لمولاس البنجر ، وفهلنج لمولاس القصب ) بمعدل ٠,٢١ % – ٧,٤١ % على التوالي ، ويرجع ذلك الى المقدرة الاختزالية لبعض المواد العضوية وغير العضوية الموجودة بالمولاس والتي تتداخل مع السكريات المختزلة.

وتراوحت النسبة بين الجلوكوز والفركتوز بطريقة HPLC بين ٠,٦٨ : ١ في عينات مولاس القصب بمتوسط ٠,٧٧ : ١ ، الى ١,١٨ : ١ في عينات مولاس البنجر بمتوسط ٠,٩٣ : ١ مقارنة بالطرق التقليدية المعروفة نتائجها لدينا ١ جلوكوز : ١ فركتوز.

وقد تحقق من النتائج أن تقدير السكريات بطريقة HPLC يكون سريع ، ودقيق ، ويمكن تكراره لتأكيد نتائج التحليل. وتعتبر طريقة هامة جدا لحساب فقد السكر بالمولاس النهائي ، وكذلك تقدير المولاس.