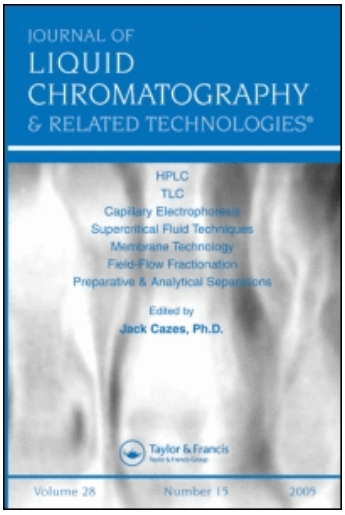

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A RAPID DETERMINATION OF SUCROSE AND FRUCTOSE IN BIOLOGICAL SAMPLES BY VIDEO DENSITOMETRY

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ABSTRACT

Simple, rapid methods which have been developed for the separation and quantification of sucrose and fructose in biological materials are presented. The method uses thin layer chromatography. The saccharides are stained with thymol-reagent on the chromatograms. The quantitative determination of red spots of saccharides are performed with a Telechrom video densitometer. The density of the spots are directly proportional to the concentration of the saccharides in a certain range. The saccharides content of the sample may be determined with a standard curve on the same chromatograms. The reproducibility of the methods are characterized by a cofficient of variation of about 4 - 5 %.

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INTRODUCTION

Determination of saccharides in biological materials is very important especially in the food industry, where the profit depends on the sugar content of biological materials, for example in sugar factories. It is essential to know the sugar or saccharides content of ready made products because of health considerations.

The classical gas-liquid chromatography utilized different derivatives of saccharides /1,2/. These methdes provide great sensivity for the detection of saccharides but they are complicated and expensive. The same when high-performance liquid chromatography techniques are used.

A simple method employs thin layer chromatography to separate the saccharides and thymol-reagent for detection and quantification by video densitometry. The method was applied to quantify the sucrose-content of sugar-beets and fructose-content of different canned fruits and jam.

REAGENTS AND MATERIALS

Merck 5554 Kieselgel G thin layer plates /10x10 cm/ were used to separate the saccharide components. For detection of spots, the chromatograms were air dried and sprayed using a solution of thymol /500 mg/ in ethanol /95 ml/ and sulfuric acid /5 ml/.

All reagents for the process were purchased from Reanal /Hungary/.

Apparatus

The spots of saccharides were quantitated by Telechrom OE 976 type video densitometer in reflection mode. The working principle of this instrument has been described /3/.

Thin layer chromatography

The standard aqueous solutions of saccharides /4-8 mg/ml/ were spotted /2-2 µl aliquots/ with a special micropipette. 2 µl of sample was applied to layers beside the spots of the standard solutions. Plates were developed at room temperature at a distance 8-8,5 cm beyond the origin line when using the following systems.

Developing system for sucrose:

1st system:

ethylacetate : 2-propanol : water - 6 : 3 : 1

Developing system for fructose:

2nd system:

2-propanol:1-butanol:0,5% boric acid in water-5:3:2

3rd system:

1-butanol:acetone:0,5% boric acid in water-4:5:1

The plates were developed to determine the sucrose in 20 minutes using the 1st system. In case of determination of fructose, at first the plates were developed in the 2nd system in 80 minutes and then the plates were air dried and developed in the 3rd system for 20 minutes.

For detection the chromatograms were air dried and stained with thymol-reagent. Every time the plates were sprayed with equal volume of thymol-reagent and then were placed in a 120-125 °C oven for 10-15 minutes.

RESULTS AND DISCUSSION

We used the 1st system to separate the sucrose from other saccharides. The R_f values and spot colors are shown in Table I.

The sucrose was detected as red spots, the color which is the most suitable for video densitometric determination with Telechrom OE 976. The calibration plot for sucrose was linear in the 8 - 16 µg range. The calibration curves /Figure 1./ were quite reproducible and linear in this range. However the value of density of the same spots is different from chromatogram to chromatogram. The value of density depends on the amount of thymol-reagent used, the temperature of the oven and the time of detection of spots. Therefore, we made a calibration curve in the same time and on the same plate with all the samples /Picture I./.

Table I.

R_f values and spot colors of some saccharides on Kieselgel G layer developed 1st system and detected with thymol-reagent.

Saccharides	R_f	Color
raffinose	0,05	red-brown
sucrose	0,13	red-brown
galactose	0,16	red
glucose	0,20	red
fructose	0,24	red
arabinose	0,30	blue
xylose	0,36	blue
rhamnose	0,45	yellow

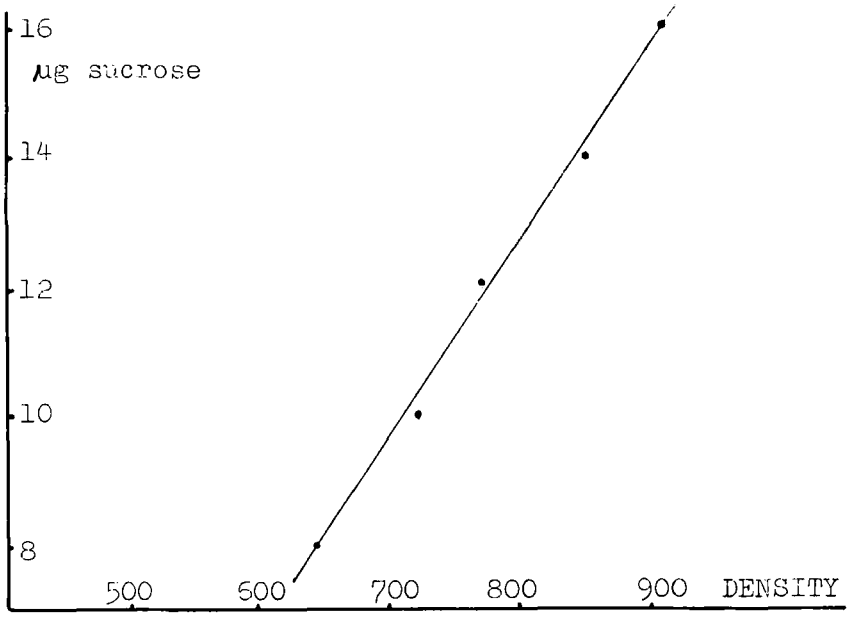
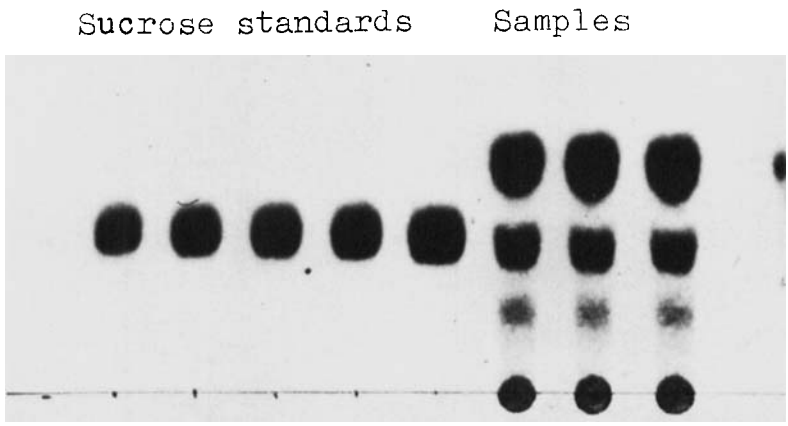


Figure 1. Calibration curve of sucrose



Picture I. The sucrose standards and samples on the chromatogram.

On the same plate we investigated the reproducibility of the method, when seven 12 ug spots of sucrose were spotted on the plate. The plate was developed, air dried, sprayed and measured with video densitometry.

The reproducibility of the method is characterized by a cofficient of variation of about 4 - 5%. In case of determination of fructose we used the 2nd and 3rd systems. In these systems R_f values are shown in Table II.

The fructose-content was determined in the same way as sucrose. The calibration plot for fructose was linear in the 8 - 16 µg range. The spot of fructose is little diffused but in spite of it the method is reproducible.

These methods were employed to quantitate of sucrose in sugar-beets before the sugar would have been removes from it, and we controled fructose-content of different bottled fruit made for diabetic patients.

Table II.

R_f values of some saccharides on kieselgel G layer. The plates were developed in the 2nd system and air dried and again developed in the 3rd system.

Saccharides	R_f /xylose/
xylose	1,00
sucrose	0,83-0,87
glucose	0,80-0,84
galactose	0,79-0,84
fructose	0,62-0,68
raffinose	0,40-0,45

SUMMARY

Thin layer chromatography with video densitometry provides rapid information on the quality of sucrose or fructose in biological samples.
Advantages of these methods are simplicity and speed. Many samples can be analysed with the standaeds in parallel.
The reproducibility of the method is suitable to determine the sucrose or fructose-content of biological materials.

REFERENCES

1. Niedermeier, W., Anal. Biochem., 40, 465 /1971/
2. Bhatti, T., Chambers, R.E. and Clamp, J.R., Biochim. Biophys. Acta, 222, 339 /1970/
3. Pongor, S., j. Liq. Chromatogr., 5, 1583 /1982/