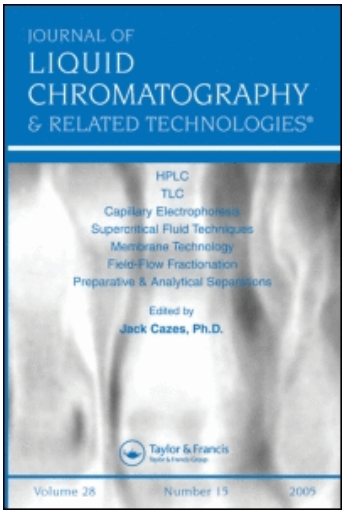

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Helthuis[ab]; T. Heidema[a]; Gorin[b]
[a] Sprenger Institute, Wageningen, Netherlands [b] Dept. of Chemistry, Tertiary Techn. Coll., Hengelo

To cite this Article Helthuis, Heidema, T. and Gorin(1981) 'Enzymatic Estimation and Quantitative High-Pressure Liquid Chromatography of Fructose, Glucose and Sucrose in Powders from Rose Petals', Journal of Liquid Chromatography & Related Technologies, 4: 8, 1401 — 1408
To link to this Article: DOI: 10.1080/01483918108059617
URL: http://dx.doi.org/10.1080/01483918108059617

JOURNAL OF LIQUID CHROMATOGRAPHY, 4(8), 1401-1408 (1981)

ENZYMATIC ESTIMATION AND QUANTITATIVE HIGH-PRESSURE LIQUID CHROMATOGRAPHY OF FRUCTOSE, GLUCOSE AND SUCROSE IN POWDERS FROM ROSE PETALS

Helthuis(1), T. Heidema and Gorin (2)

Sprenger Institute
P. O. Box 17
6700 AA Wageningen, Netherlands

ABSTRACT

There was no significant difference between mean mass fractions of fructose, glucose and sucrose measured enzymatically and by liquid chromatography. So the chromatographic method can be used on powders from rose petals as the peaks of the chromatograms are really caused by the sugars.

A method is described for clean-up of the rose extract before injecting it into the high-pressure liquid chromatograph.

INTRODUCTION

The purpose of this work was to find a way of estimating soluble sugars in cut flowers by high-pressure liquid chromatography.

As yet, we have used an enzymatic method (10). However, the enzymatic analysis had 3 disadvantages:

1. Methanol must be removed from the extract; otherwise commercial enzyme preparations are inactivated.
2. For estimation of sucrose, when the mass ratio of glucose to sucrose is higher than 3:1, glucose must be removed with glucose oxidase and catalase (11).

3 Chromatography offers more options for automation than the enzymatic method.

If the two methods gave similar results for roses, we could assume that peaks for the sugars were not due to artefacts.

EXPERIMENTAL

The roses were cut flowers of the cultivar Sonia.

The powder was prepared from petals of roses by a similar method to onion powder (8). Each sample was bulked from petals of six flowers. In total, there were 20 samples. The samples represented 6 stages of development: 1, 2, 3, 4, 5 and 6 (6) picked on 7 July 1980 (Samples 1-6 of Table 2). Stages 1 and 3 were picked on 13 July 1980 (Samples 7 and 14 of Table 2). The chemical analyses formed part of a study on longevity of the blooms on the plant and after cutting:

Stage 1: 3 days on the plant (Samples 11, 12 and 13); 3 days in a vase (Samples 8, 9 and 10) .

Stage 3: 3 days on the plant (Samples 18, 9, 10); 3 days in a vase (Samples 15, 16 and 17).

Fructose, glucose and sucrose in powders were estimated as described (10) with 80% buffered methanol (0.2 mol/l acetate buffer pH 6.5) and using 0.1 g powder plus 10 ml of buffered methanol for extraction of sugars. For chromatography, the ratio of powder to the methanol was 10 times as great, but extraction of sugars was not affected.

The NADP+ solution was prepared freshly every 3 days (3) and not every month(4).

For chromatography, the buffered methanol was prepared from 20 mL of triethanolamine hydrochloride buffer (pH 7.0) of concentration 0.1 mol/L (4) and 80 mL of absolute methanol. The final concentration of the buffer in the 80% methanol was 0.02 mol/L. The pH, measured with an indicator strip, was

TABLE 1. Enzymatic analysis of soluble sugars in rose powder with or without clean-up of the extract. Values are mass fraction of sugar (mg per 100 mg).

SAMPLE NO.	GLUCOSE				FRUCTOSE			
	Without Clean-up		With Clean-up		Without Clean-up		With Clean-up	
	$\bar{x}_1$	s_1	$\bar{x}_2$	s_2	$\bar{x}_1$	s_1	$\bar{x}_2$	s_2
7	1.17	0.04	1.27	0.03	1.05	0.03	1.16	0.01
8	0.89	0.03	0.82	0.02	0.93	0.02	0.94	0.02
9	2.07	0.01	1.95	0.03	2.21	0.04	2.17	0.06
10	2.85	0.05	2.96	0.05	3.01	0.07	3.10	0.08
11	1.29	0.03	1.29	0.02	1.41	0.02	1.46	0.02
12	2.33	0.07	2.42	0.07	2.67	0.08	2.50	0.05
13	6.63	0.13	6.40	0.12	5.74	0.15	5.99	0.10

$\bar{x}_1$ and $\bar{x}_2$ are averages of triplicate analysis. $s_1 = s_2 = (\Sigma\, d^2/n-1)^{\frac{1}{2}}$

TABLE 2. Mass fraction of glucose, fructose and sucrose in rose powder (mg per 100mg) as estimated enzymatically and by HPLC.

SAMPLE NO.	GLUCOSE				FRUCTOSE				SUCROSE			
	Enzymatic		Chromatographic		Enzymatic		Chromatographic		Enzymatic		Chromatographic	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
1	0.82	0.02	0.81	0.01	0.71	0.02	0.81	0.01	0.57	0.02	0.51	0.01
2	1.31	0.02	1.22	0.01	1.58	0.02	1.58	0.01	0.64	0.03	0.65	0.01
3	0.91	0.01	0.91	0.01	1.17	0.03	1.23	0.01	0.43	0.02	0.45	0.01
4	1.58	0.03	1.55	0.01	1.20	0.04	1.31	0.01	0.71	0.02	0.80	0.01
5	5.27	0.10	5.18	0.05	7.61	0.08	7.39	0.06	2.91	0.08	2.75	0.03
6	8.38	0.10	8.17	0.06	10.88	0.10	10.64	0.07	0.79	0.03	0.82	0.02
7	1.17	0.04	1.22	0.03	1.05	0.03	1.05	0.02	0.81	0.02	0.67	0.04
8	0.89	0.03	0.92	0.02	0.93	0.02	0.90	0.02	0.20	0.01	ND[2]	
9	2.07	0.01	2.15	0.05	2.21	0.04	2.17	0.03	ND[1]		ND[2]	
10	2.85	0.05	2.80	0.02	3.01	0.07	3.01	0.03	ND[1]		ND[2]	
11	1.29	0.03	1.24	0.02	1.41	0.02	1.48	0.02	0.41	0.01	0.43	0.04
12	2.33	0.07	2.47	0.04	2.67	0.08	2.73	0.02	0.57	0.02	0.52	0.04
13	6.63	0.13	6.91	0.05	5.74	0.15	5.68	0.06	0.42	0.01	ND[2]	
14	1.93	0.03	2.00	0.01	2.28	0.05	2.33	0.01	0.40	0.02	0.31	0.02
15	4.16	0.02	4.07	0.04	5.30	0.26	5.20	0.02	0.23	0.01	ND[2]	
16	5.02	0.08	5.07	0.03	5.30	0.06	5.48	0.04	ND[1]		ND[2]	
17	6.23	0.09	6.10	0.04	7.01	0.15	7.22	0.04	ND[1]		ND[2]	
18	3.64	0.15	3.72	0.04	4.68	0.17	4.65	0.03	0.48	0.02	0.49	0.02
19	7.97	0.25	8.14	0.18	10.73	0.26	10.46	0.15	0.33	0.01	ND[2]	
20	13.64	0.30	13.48	0.06	17.62	0.40	17.68	0.17	0.43	0.01	ND[2]	

ND[1] Not detected. Value less than 5/μg sucrose in the cuvette (enzymatic analysis).

ND[2] Not detected. Value below 18-25 μg sucrose in 20 μl soln, when injected into the liquid chromatograph.

$\bar{x}$ = average of triplicate analysis, $s = (\Sigma d^2/n-1)^{\frac{1}{2}}$

between 6.5 and 7.0. This type of buffer did not interfere with peaks for sugars, giving a peak in the chromatogram before those of the sugars.

Buffered methanol with acetate (9) was not suitable because we had to increase the concentration from 0.05 to 0.2 mol/L in order to maintain the pH of the extract between 6.0 and 6.5. When the concentration was increased, a peak of acetate appeared between glucose and sucrose.

A buffer of 80% methanol with β , β'-dimethylglutaric acid-NaOH (pH 6.8) (5) of final concentration 0.05 mol/L held the pH of the extract between 6.0 and 6.5, but produced a concavity in the base line of the chromatogram that interfered with the peak of sucrose.

Rose extract was prepared from 1 g powder and 10 ml buffered methanol (previously kept at 20°C) poured into a Pyrex centrifuge tube and placed in a water bath at 55°C.

After 15 min., the suspension was spun for 40 min. at 1600 g at 6°C. The supernatant was the rose extract; its pH was kept between 6.0 and 6.5 (to avoid hydrolysis of sucrose), as checked with a strip indicator.

To clean up the rose extract (whose weight was estimated and of volume about 9mL), it was suspended together with 100 mg of Polyclar AT (BDH, art. 44201) and vibrated (Vibro-Mixer, Vortex Genie) for 1 min.; active charcoal, Darco G 60 (Fluka, A.G. Buchs SG, art. 05100), was added to a mass ratio to the initial rose extract of 0.01.

The suspension was again vibrated for 1 min. and spun at 1600 g for 30 min. at 6°C. The supernatant was collected and weighed. If the supernatant was still colored, a new portion of active charcoal was added to a mass ratio of 0.01, shaken and spun.

The supernatant, colorless at first sight, was passed through a minicolumn Sep-Pak C18, (Waters, art. 51910), and a minicolumn of Al_2O_3 (9).

The cleaned extract was filtered through Millipore filter FHLP 01300 to remove any particles of glass wool and was treated in an ultrasonicator to remove dissolved air.

A reference solution containing fructose, glucose and sucrose was submitted to the same clean-up procedure. Peaks were the same as without clean-up. Recovery was 97 ± 1% for fructose, 100 ± 1% for glucose and 98 ± 1% for sucrose.

Under ultraviolet radiation (wavelength 360 nm), the Al_2O_3 minicolumn with reference solution was uniform in violet color whereas those with samples contained bands of several colors.

Cleaned extracts poured into an Al_2O_3 minicolumn were also uniform, showing that extraneous materials had been removed.

Chromatography was as described by Gorin and Heidema (9) but the cleaned extract was injected automatically (20 µL instead of 10 µl) with the WISP (Waters Intelligent Sampling Processor 710A), instead of manually.

As spiking procedure, fructose (Merck, art. 5323) (5.00 mg), glucose (Merck, art. 8342) (5.01 mg) and sucrose (Merck, art. 7651) (5.00 mg) were added separately to 100 mg of powder and measured by the procedures. The recoveries were 102 ± 2% for glucose, 101 ± 1% for fructose and 99 ± 1% for sucrose.

Fructose and glucose were also measured enzymatically in several powders with and without clean-up.

Sucrose was not measured because if the sample had a mass ratio of glucose to sucrose >3:1, natural glucose had to be destroyed by a tedious procedure.

Both types of extracts were diluted to a tenth with distilled water and poured into the cuvette (where there was a further dilution by a factor 30). A total dilution of 300 times "removed" methanol and also the pigments of the samples without clean-up (8).

The extract could be diluted 10 times, because they originated from 1.0 g powder in this series of experiments instead of 0.1 g and so provided sufficient sugars for the sensitivity of the method.

Moisture in powders was estimated by the method of Gorin (7).

RESULTS AND DISCUSSION

Response of Detector

There was a rectilinear response between peak height (cm) and mass of soluble sugars of 25, 50, 75 and 100 ug in 20 uL. The equation ($y=a_o+a_1x$) of the 3 lines are:

for fructose $a_o = -0.400$, $a_1 = 0.251$, $r= 1.000$

for glucose $a_o = -0.315$, $a_1 = 0.202$, $r= 1.000$

for sucrose $a_o = -0.215$, $a_1 = 0.190$, $r= 1.000$

Enzymatic Comparison With and Without Clean-up

A sign test for paired comparisons (12) showed no significant difference between mean mass fractions of the respective sugars with and without clean-up (Table 1). So the clean-up procedure did not retain the sugars of the samples.

Comparison of Enzymatic and Chromatographic Method

The sign test for paired comparisons (12) demonstrated that there was no significant difference between the mean mass fractions of the respective sugars by the two methods.

So peaks of the chromatograms for the powders were due to fructose, glucose, and sucrose. Other compounds did not interfere.

The mass ratio of powder to methanol buffer (1:10) did not constitute a problem for extraction.

The moisture contents (between 0.5 and 3.0%) of the powders were ignored and not corrected for.

ACKNOWLEDGEMENT

We thank Hendrik Zonneveld for his idea of aluminium oxide minicolumns, Richardus A. Hilhorst for statistical calculations and J. Christopher Rigg (Centre for Agricultural Publishing and Documentation, Wageningen) for help in drafting the manuscript.

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