

## Simultaneous gas–liquid chromatographic determination of sugars and organic acids as trimethylsilyl derivatives in vegetables and strawberries

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### ABSTRACT

The simultaneous determination of organic acids (succinic, malic, pimelic, tartaric, shikimic, citric, quinic, caffeic and chlorogenic acids), sorbitol and sugars (arabinose, rhamnose, fructose, galactose, glucose, sucrose, maltose and raffinose) as trimethylsilyl (TMS)-oxime and TMS derivatives in a single solution has been developed. The optimum conditions for the simultaneous derivatization and the rapid quantitative evaluation of the nineteen compounds by capillary gas–liquid chromatography are reported in this paper. Reproducibility data reveal that  $\geq 0.02 \mu\text{g}$  of acids in the presence of  $\leq 12 \mu\text{g}$  of sugars can be determined with a relative standard error (R.S.E.) of  $\leq 11.2\%$ , whereas the main constituents are determined with an R.S.E. of  $\leq 3.6\%$ .

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### INTRODUCTION

It is important to be able to determine, both qualitatively and quantitatively, the composition of organic acids and sugars present in plant foods. The concentrations of these compounds in vegetables or fruits are characteristic, and are influenced by factors such as maturity, ripeness and storage conditions. The recommended analytical methods for the determination of sugars and organic acids are often used in parallel, rather than simultaneously. However, some methods have recently been published which can be used to determine sugars and organic acids simultaneously. These methods include high-performance liquid chromatography (HPLC) of apple juice [1] and in cucumber fermentation [2], and gas–liquid chromatography (GLC) in grape musts [3], propolis [4], birch sap [5] and apple samples [6].

The goal of the work reported here was to extend earlier work [6–10] on the simultaneous determination of sugars and organic acids in carrots, potatoes, cucumbers and strawberries. These compounds are present in very different concentra-

tions. A second goal was to complete the recently [6] developed technique for a simple and rapid capillary GLC procedure.

## EXPERIMENTAL

All materials and reagents were of analytical-reagent grade from Reanal (Budapest, Hungary), Fluka (Buchs, Switzerland) and Serva (Heidelberg, Germany).

Carrots, cucumbers and potatoes were purchased from the local market. Strawberries of known varieties were obtained from the Research Farm of the University of Horticulture and Food Industry, Horticulture Faculty (Kecskemét, Hungary). The varieties used were as follows: (1) Induka; (2) Korona; (3) Kortés; (4) Primella; (5) Pocahontas; (6) Tufts; (7) Aiko; (8) Aliso; (9) Elvira; (10) Favorit; (11) Fertődi; and (12) Gorella.

### *Preparation of the samples for gas-liquid chromatographic analysis*

Peeled carrot, cucumber and potato were grated and 2, 5, 10 or 20 g of the homogenised samples were either extracted at 25°C or by refluxing with 80% aqueous methanol or ethanol for 20 min. The insoluble material was then collected on a glass filter (6 cm I.D.) and washed several times with 80% aqueous methanol or ethanol. The combined filtrates and washings were concentrated to 100 cm<sup>3</sup> (stock solution) on a rotary evaporator at 50–60°C.

The strawberries were homogenised in a food mixer and the sieved pulp (free of stones) was used for derivatization.

### *Preparation of trimethylsilyl (TMS) oxime and TMS derivatives*

Various amounts of organic acids, arabinose, rhamnose, sorbitol, maltose or raffinose ( $1.25 \cdot 10^{-4}$  –  $1 \cdot 10^{-3}$  g) and the same amount of sugars ( $2.5 \cdot 10^{-3}$  –  $2 \cdot 10^{-2}$  g of fructose, glucose and sucrose) in model solutions (weighed with analytical precision) or the stock solutions of vegetables or strawberry pulps, (containing sugars and acids corresponding to the amount applied in standards) were evaporated to dryness in a rotary evaporator at 50–60°C. The dehydrated residues were treated with 1.0 cm<sup>3</sup> of pyridine (containing 1.25 g of hydroxylamine hydrochloride per 100 cm<sup>3</sup>) and were heated for 30 min at 75°C. The cooled samples were then trimethylsilylated with a mixture of 1.8 cm<sup>3</sup> of hexamethyldisilane (HMDS) and 0.2 cm<sup>3</sup> of trifluoroacetic anhydride (TFAA) in 4-cm<sup>3</sup> reactivials for 60 min at 100°C. The filtered solutions were then evaporated to 0.5 cm<sup>3</sup> in a rotary evaporator at 50–60°C. The residues were transferred quantitatively to 1-cm<sup>3</sup> reactivials with a solution of HMDS–TFAA (9:1) and diluted to a volume of 1 cm<sup>3</sup>.

### *Separation of TMS-oxime or TMS derivatives*

The gas chromatograph used was a Chrompack CP 900 instrument (Chrompack International, Middelburg, The Netherlands) equipped with a flame ionisation detector and a wall-coated open tubular fused-silica column (10 m × 0.25 mm I.D.), coated with CP-Sil-5CB, DF 0.12 (Chrompack, Cat. No. 7700, column No. 225660).

The temperatures of both the injection and detector ports were 300°C. Using a temperature programme of 120–160°C at 10°C/min and a hold-time at 160°C of 3 min, followed by 160–280°C at 10°C/min and a hold-time at 280°C of 6 min, 30 min were required to elute the TMS-oxime and TMS derivatives of the organic acids and sugars. The nitrogen pressure was 30 kPa with a split ratio of 100:1.

## RESULTS AND DISCUSSION

*Extraction, derivatization and gas-liquid chromatographic conditions*

**Extraction.** A quantitative extraction procedure is the first step in obtaining accurate acid and sugar determinations by GLC analysis. A study has been carried out to determine the optimum extraction conditions (Fig. 1a-d: extraction yield of carrot), as the available literature data [11-14] were contradictory in two respects. These are: (1) the low ratio of the extracting agent to the sample [11-14] *e.g.* applying 10 [12], 14 [14], 20 [13] or 50 g [11] of sample to 100 cm<sup>3</sup> of 60 [11], 75 [13] or 80% [12,14] aqueous ethanol; and (2) the time and temperature conditions of extractions: *e.g.* performing the extractions for 5 [11] or 10 min [13] at room temperature, or overnight at 0°C [14], or by boiling for 15 min [12].

The extractions carried out, which were followed by measuring the main acid and sugar components of carrots, potatoes and cucumbers as TMS-oxime and TMS derivatives, showed that quantitative extraction yields can be achieved using 2 or 5 g of sample per 100 cm<sup>3</sup> of 80% aqueous methanol or ethanol at 25°C, or by refluxing with

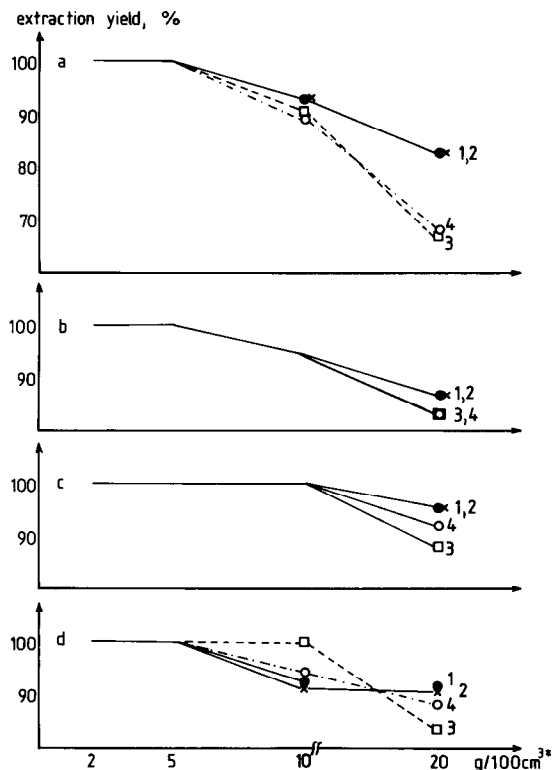


Fig. 1. Extraction yields of the main components of carrot, obtained under various conditions. (a) Malic acid; (b) fructose; (c) glucose; (d) sucrose. Conditions: addition of (1) 2; (2) 5; (3) 10; and (4) 20 g of fresh grated carrot to 100 cm<sup>3</sup> of 80% aqueous methanol (1,2) or 80% aqueous ethanol (3,4) with continuous stirring for 20 min. (1,3) Values obtained with extractions at 25°C; (2, 4) heated under refluxing. Results expressed as g per 100 cm<sup>3</sup>\* relative to the weight of fresh carrot.

stirring for 20 min. This was achieved for all four components (Fig. 1a–d, malic acid, fructose, glucose and sucrose extraction yields of carrot). A shorter extraction time (10 min) or lower ratio of sample to extracting agent (10 or 20 g per 100 cm<sup>3</sup>) was unsatisfactory. The same trend of extractions were observed for potatoes and cucumbers.

*Derivatization and gas-liquid chromatographic conditions.* To obtain a simultaneous quantitative derivatization, without any side-reactions, of the organic acids and sugars, a detailed study was required based on earlier studies [6,15] with packed columns (15% Dexsil 300 GC, 3 m × 4 mm I.D.).

In this work the basic investigations of derivatization were repeated. The data obtained (by measuring the products on the capillary column) have proved that using this derivatization procedure and a method with about a five-fold increase of sensitivity, and comparing the results to those obtained using a packed column, no side-reactions could be detected.

To determine the optimum GLC conditions, and taking into account the literature data [16], the 10-, 20- and 30-m columns and several temperature programmes were tested. A 10-m capillary column and the temperature programme detailed under experimental was determined to be the optimum for this purpose.

TABLE I

REPRODUCIBILITY OF THE SIMULTANEOUS DETERMINATION OF VARIOUS AMOUNTS OF ORGANIC ACIDS AND SUGARS AS TMS-OXIME OR TMS DERIVATIVES BY CAPILLARY GLC

The letters a–d define the amounts of components injected: (a) 0.125, (b) 0.25, (c) 0.60 and (d) 1.0 µg of organic acids or arabinose, rhamnose, maltose or raffinose, and (a) 0.25, (b) 5, (c) 10 and (d) 20 µg of fructose, glucose or sucrose.

Acid/sugar	Integral units equivalent to 1 µg of substance					Standard error	Relative standard error (%)
	a <sup>a</sup>	b <sup>a</sup>	c <sup>a</sup>	d <sup>a</sup>	Mean <sup>b</sup>		
Succinic acid	(1944)	1385	1318	1396	1366	68.7	5.0
Malic acid	(2338)	1784	1848	1796	1809	34.0	1.9
Pimelic acid	(1975)	1542	1548	1500	1530	26.1	1.7
Tartaric acid	2451	2423	2638	2482	2499	96.1	3.8
Arabinose	3347	3390	3180	3249	3304	96.0	2.9
Shikimic acid	2632	2694	2716	2708	2688	38.1	1.4
Citric acid	1997	1995	1957	1942	1973	27.0	1.4
Rhamnose	2389	2400	2376	2503	2417	58.2	2.4
Quinic acid	2924	2980	2944	2955	2958	23.4	0.79
Sorbitol	3967	4205	4172	3913	4064	145.8	3.6
Fructose	3383	3370	3302	3246	3325	63.7	1.9
Glucose	3606	3672	3535	3664	3619	63.4	1.8
Caffeic acid	2444	2525	2501	2448	2480	39.9	1.6
Sucrose	3184	3109	3188	3160	3160	36.3	1.2
Maltose	3073	3078	3107	2987	3061	51.7	1.2
Chlorogenic acid	3711	3814	3837	3727	3772	62.5	1.7
Raffinose	2884	2972	3160	2967	2996	116.7	3.9

<sup>a</sup> Mean of at least three parallel tests.

<sup>b</sup> Mean of a–d values.

TABLE II  
REPRODUCIBILITY OF SUGAR AND ORGANIC ACID COMPONENTS OF CARROTS, POTATOES AND CUCUMBERS OBTAINED AFTER QUANTITATIVE EXTRACTION (TESTS a-d) AND OF STRAWBERRIES, MEASURED AS TMS-OXIME OR TMS DERIVATIVES

Acid/sugar	Composition <sup>a</sup> (% w/w)						R.S.D. (%)		
	Carrots			Strawberries					
	a	b	c	d	Mean $\pm$ S.D.	Potatoes (test a)		Cucumbers (test a)	Strawberries
Succinic acid	0.078	0.093	0.085	0.091	0.087 $\pm$ 0.007	0.0135	7.8	0.0035	0.0070
Malic acid	0.59	0.59	0.58	0.58	0.58 $\pm$ 0.008	0.068	1.3	0.17	0.036
Pimelic acid	0.028	0.031	0.031	0.028	0.029 $\pm$ 0.002	0.044	6.3	—	—
Tartaric acid	0.0067	0.0068	0.0054	0.0070	0.065 $\pm$ 0.00072	0.017	11.2	0.011	—
Pentose	— <sup>b</sup>	—	—	—	—	—	—	—	0.038
Citric acid	0.071	0.073	0.066	0.066	0.069 $\pm$ 0.0036	0.045	5.2	—	0.438
Quinic acid	0.10	0.10	0.10	0.10	0.10	—	—	—	0.0066
Sorbitol	0.015	—	—	—	—	—	—	—	—
Fructose	0.89	0.89	0.88	0.93	0.89 $\pm$ 0.024	0.67	2.7	0.99	1.98
Glucose	1.09	1.01	1.09	1.08	1.07 $\pm$ 0.039	0.53	3.6	0.99	1.74
Caffeic acid	0.067	0.069	0.074	0.070	0.070 $\pm$ 0.0029	0.035	4.2	0.043	0.049
Sucrose	1.67	1.77	1.75	1.74	1.76 $\pm$ 0.014	0.55	0.8	0.023	0.75
Maltose	—	—	—	—	—	0.0033	—	—	0.0030
Chlorogenic acid	0.0030	—	—	—	—	—	—	—	—
Raffinose	0.019	0.021	0.020	0.024	0.021 $\pm$ 0.0021	—	1.0	—	0.0085

<sup>a</sup> Calculated as the percentage of the fresh weight of samples a-d carried out in parallel. The mean values obtained after eight quantitative extractions, *i.e.* 2 or 5 g of vegetable, using 100 cm<sup>3</sup> of 80% aqueous methanol (tests a and b) or 100 cm<sup>3</sup> of aqueous ethanol (tests c and d). a and c were extracted at 25°C; b and d were extracted using reflux condenser.

<sup>b</sup> No data available, *i.e.* values below detection limit.

*Reproducibility study*

The reproducibility and usefulness of the proposed method were shown by the results with model solutions (Table I, Fig. 2a) and by the composition of carrots (Table II, Fig. 2b). The reproducibility measurements showed a relative standard error (R.S.E.) of 0.8–11.1% (depending on the amount used and the detection limit of the minor constituents). For the major components such as malic acid, fructose, glucose and sucrose, an R.S.E.  $\leq$  3.6% was obtained.

TABLE III

## DISTRIBUTION OF THE MAIN CHARACTERISTIC COMPONENTS OF STRAWBERRIES (VARIETIES 1–12) AS A FUNCTION OF RIPENESS

Gathered on 16 May 1990 (a), on 24 May 1990 (b) and on 31 May 1990 (c).

Varieties	Time of gathering	Distribution (% , w/w)				
		Malic acid	Citric acid	Fructose	Glucose	Sucrose
Induka	a	0.213	0.492	1.73	1.54	0.323
	b	0.098	0.506	1.77	1.58	0.220
	c	0.027	0.327	2.88	2.58	0.144
Korona	a	—	0.054	2.05	1.72	0.302
	b	0.013	0.505	2.05	1.82	0.458
	c	0.018	0.358	3.43	3.02	0.421
Kortes	a	0.018	0.660	1.81	1.67	0.545
	b	0.017	0.573	2.09	1.88	0.387
	c	0.034	0.290	2.70	2.38	0.400
Primella	a	0.021	0.682	2.35	2.22	0.035
	b	0.045	0.598	2.35	1.98	0.15
	c	0.067	0.278	2.61	2.39	0.246
Pocahontas	a	0.076	0.559	1.96	1.66	0.180
	b	0.016	0.393	2.58	2.25	0.028
	c	0.016	0.389	1.84	1.72	—
Tufts	a	0.045	0.321	1.65	1.46	0.132
	b	0.049	0.343	2.47	2.19	0.111
	c	0.016	0.335	3.01	2.56	0.022
Aiko	a	0.044	0.458	1.73	1.42	0.479
	b	0.045	0.495	1.74	1.55	0.592
	c	0.036	0.438	1.98	1.74	0.75
Aliso	a	0.050	0.837	1.71	1.51	0.36
	b	0.028	0.371	2.04	1.91	0.093
	c	0.0025	0.321	3.08	2.81	—
Elvira	a	0.068	0.913	2.10	1.95	0.265
	b	0.064	0.888	2.50	2.21	0.029
	c	—	0.042	2.50	2.31	0.013
Favorit	a	0.092	0.726	1.50	1.47	0.34
	b	0.054	0.477	2.07	1.81	0.089
	c	0.0015	0.010	2.79	2.62	—
Fertődi	a	0.189	0.156	1.48	1.30	0.016
	b	0.111	0.332	1.81	1.51	—
	c	0.096	0.215	2.15	1.72	—
Gorella	a	0.029	0.387	1.74	1.54	0.031
	b	0.034	0.487	2.64	2.38	0.010
	c	0.012	0.422	2.02	1.77	—

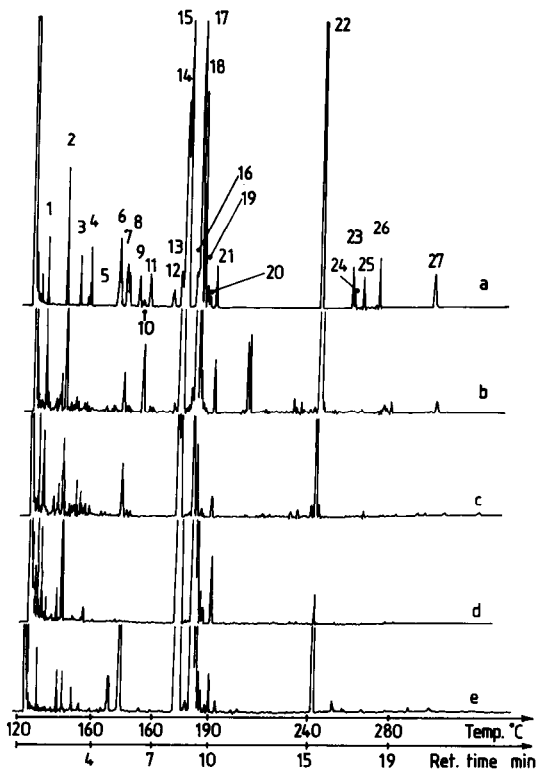


Fig. 2. Chromatograms of TMS and TMS-oxime derivatives of acids and sugars obtained with (a) model solutions, (b) extracts of carrot, (c) potato, (d) cucumber and (e) strawberry pulp (variety 1). Peaks: 1 = succinic acid; 2 = malic acid; 3 = pimelic acid; 4 = tartaric acid; 5 = xylose; 6 = arabinose; 7 = shikimic acid; 8 = citric acid; 9 = rhamnose<sub>1</sub>; 10 = rhamnose<sub>2</sub>; 11 = quinic acid; 12 = fructose<sub>1</sub>; 13 = sorbitol; 14 = fructose<sub>2</sub>; 15 = fructose<sub>3</sub>; 16 = galactose; 17 = glucose<sub>1</sub>; 18 = glucose<sub>2</sub>; 19 = glucose<sub>3</sub>; 20 = glucose<sub>4</sub>; 21 = caffeic acid; 22 = sucrose; 23 = maltose<sub>1</sub>; 24 = maltose<sub>2</sub>; 25 = chlorogenic acid<sub>1</sub>; 26 = chlorogenic acid<sub>2</sub>; 27 = raffinose. Note: the elimination of peaks 12, 19 and 20, probably originating from incomplete oximation, needs further study.

### *Composition of vegetables and strawberries*

The sugar and organic acid contents of carrots, potatoes, cucumbers and strawberries were measured as TMS-oxime and TMS derivatives (Tables II and III and Fig. 2b-e). No literature data could be found for the GLC analysis of these components from a single injection using the same varieties of strawberries. The evaluation of this extraction and GLC procedure was therefore restricted. However, it was shown to serve as a general application both for vegetables extracted prior to derivatization and for directly derivatizable pulps, such as strawberries.

The data showed that constituents from  $3 \cdot 10^{-3}\%$  (Table II, 0.003% chlorogenic acid) to about 1.8% (Table II, 1.76% sucrose) could be determined with an acceptable reproducibility.

The usefulness of the method can be characterised by the determination of the

small changes in both the organic acid and the sugar composition of the different varieties of strawberries obtained as a function of maturity (Table III).

The comparison of these results with previously published values is limited by: (1) the basis of reference, which is not uniformly accepted; (2) the fact that comprehensive studies cannot be found in the literature for the optimum (quantitative) extraction and analytical conditions for the simultaneous determination of organic acids and sugars from any matrices; and (3) the compilation [17] which relates to the sugar contents of various berries from 1969 to 1979 is the only basis of comparison for strawberries. In order of listing, the fructose (1.02–3.20 g per 100 g), glucose (1.48–3.40 g per 100 g) and sucrose (0.20–1.56 g per 100 g) contents of eight varieties [originating from the U.S.A. (five), Switzerland (one), the U.K. (one) and India (one), all calculated as g per 100 g fresh weight], were in good agreement with the results obtained here for the twelve varieties grown in Hungary (Table III).

Further extension of this method to prove its use in the analysis of other natural matrices containing additional organic acid and sugar components is in progress.

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