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Short communication

Optimization of the simultaneous determination of acids and sugars as their trimethylsilyl(oxime) derivatives by gas chromatography–mass spectrometry and determination of the composition of six apple varieties

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Abstract

A GC–MS method is reported for establishing the reproducibility of the determination of widely different amounts of sugars and acids as their trimethylsilyl derivatives simultaneously, from one solution with one injection. Optimum conditions were achieved on a 30-m DB-5 column. The determination of the components was based on their TIC and on selected ion monitoring. Data furnished by a Varian Saturn II GC–MS system equipped with a Varian Model 8200 AutoSampler showed that 4–20 ng of the minor constituents, in the presence of 50–250 ng of the main components, could be determined with a relative standard deviation of 10.6% or less. The utility of the procedure was demonstrated by the analysis of the composition of six different apple varieties, gathered at three different time of ripeness, in two consecutive years (1991, 1992), and stored for various periods of time. The separated carboxylic acids and sugars were phosphoric, succinic, pyruvic, 5-hydroxy-N-valeric and malic acid, butanal, 3-methyl-2-hydroxy-2-butenoic acid, 1,2-hydroxycyclohexene, pimelic acid, 2-deoxy-D-erythrose, tartaric acid, xylitol, arabinose, caffeic acid, D-ribose, citric acid, rhamnose, quinic acid, D-*erythro*-tetrafuranose, talose, 2-ketogluconic acid, mannitol, sorbitol, fructose, galactose, glucose, fructose (open form), glucaric and galacturonic acid, lactose, *meso*-inositol, gluconic, linoleic, glucuronic, stearic and arachidic acid, sucrose, turanose, maltose, chlorogenic acid, β-sitosterol, raffinose and maltotriose.

1. Introduction

The importance of knowing the qualitative and quantitative distribution of organic acids and sugars present in fruits and vegetables and in their different products is well known. The concentrations of these compounds in fruits and vegetables are characteristic, and are influenced by a number of factors, such as variety, maturity, ripeness and storage conditions.

Optimum possibilities for the simultaneous derivatization and gas chromatographic (GC) determination of more than 30 components present in several fruits and vegetables, (on both packed and capillary columns) have been reported recently [1–8].

In studies of the simultaneous determination of sugars and acids (monitored by GC–MS as

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their TMS derivatives), Chapman and Horváth [9] determined four acids and eight sugars in extracts of apple, peach, pear and sweet potatoes, and Maciejewicz *et al.* [10] found four phenolic acids, six sugars and glucitol in the extract of propolis.

The aim of this paper is to show the extended possibilities of the simultaneous determination of sugars and acids as their trimethylsilyl derivatives, present in widely different concentrations, in one solution, by one injection, performing mass spectrometric detection with six apple varieties.

2. Experimental

2.1. Materials, reagents and samples

Chemicals and reagents were of analytical-reagent grade. Pyridine and hydroxylamine hydrochloride and model sugars and acids were obtained from Reanal (Budapest, Hungary), hexamethyldisilazane from Fluka (Buchs, Switzerland) and trifluoroacetic acid from Serva (Heidelberg, Germany).

Authentic apple varieties (Jonnee, Jonagold, Jonathan, Redspur, Gloster and Mutsu) were obtained from the Research Garden of the University of Horticulture and Food Industry (Péterimajor, Hungary). All six varieties were gathered at three different stages ($Jonnee_{1-3}$–$Mutsu_{1-3}$) of ripeness, in two consecutive years (1991, 1992), in order of listing at 03.09.91 and 03.09.92 ($Jonnee_1$–$Mutsu_1$), at 13.09.91 and 13.09.92 ($Jonnee_2$–$Mutsu_2$) and at 23.09.91 and 23.09.92 ($Jonnee_3$–$Mutsu_3$). Analyses were performed immediately after gathering (O tests), and every succeeding month, three times (A, B and C tests). Peeled apples were homogenized in a mixer and the sieved pulps were used for derivatization.

2.2. Preparation of the TMS-oxime and TMS derivatives

Model solutions containing various amounts of minor components ($5 \cdot 10^{-5}$–$2.5 \cdot 10^{-4}$ g) and main constituents ($0.5 \cdot 10^{-3}$–$5 \cdot 10^{-3}$ g of malic acid, glucose, fructose and sucrose), and stock solutions of apple pulps (containing approximately the corresponding amounts of acids and sugars, *i.e.*, 0.2–0.5 g wet samples) were evaporated to dryness in a rotary evaporator at 50–60°C using 2- or 4-ml reaction vials. The dehydrated residues were then derivatized in the same reaction vials. First they were treated with 0.5 ml of pyridine (containing 1.25 g of hydroxylamine hydrochloride per 100 ml) and were heated for 30 min at 75°C. The cooled samples were then trimethylsilylated with a mixture of 0.9 ml of hexamethyldisilazane (HMDS) and 0.1 ml of trifluoroacetic acid (TFAA), in the same vials for 60 min at 100°C.

Thereafter the solutions were ready for the analysis and could be kept at ambient temperature for at least 3 months in their initial condition. The amounts of stock solutions injected for GC–MS were the variously (10–50-fold) diluted aliquots of the derivatized stock solutions.

Table 1
Optimum parameters for GC–MS measurements

Column temperature programme

Start (°C)	End (°C)	Rate (°C/min)	Time (min)
60	120	16.0	3.75
120	155	4.0	8.75
155	155	0.0	12.00
155	210	4.0	13.75
210	320	16.0	6.87
320	320	0.0	18.00

Injector temperature programme

Start (°C)	End (°C)	Rate (°C/min)	Time (min)
60	60	0.0	2.00
60	320	180.0	1.44
320	320	0.0	10.00

Actual atomatic set-up conditions

Mass range, 40–650 u; Time per scan, 0.55; acquisition time, 60 min; Fil/Mul delay (time at the beginning of the elution, that data acquisition does not work) 420 s; peak threshold, 0 count; mass defect, 100 mu per 100 u; background mass, 50 u

2.3. Separation of the TMS-oxime and TMS derivatives

The apparatus was a Saturn II GC–MS system from Varian (Walnut Creek, CA, USA), equipped with a Varian 8200 AutoSampler and a septum-equipped programmable injector (SPI). A DB-5 (0.25 mm) column (30 m × 0.248 mm I.D.) was obtained from J & W Scientific (Folsom, CA, USA). Four other columns were obtained from Chrompack (Middelburg, Netherlands): CPF-Sil 5 CB (0.12 μm) (10 m × 0.25 mm I.D. and 25 m × 0.25 mm, I.D.) and CP-Sil 19 CB (0.2 mm) (10 m × 0.32 mm I.D. and 25 m × 0.32 mm I.D.). The temperature programmes for the columns and for the SPI are given in Table 1. The temperature of the transfer line was 300°C. The actual parameters of the ion-trap detector (ITD) were defined by the automatic set-up mode (Table 1).

3. Results and discussion

In order to utilize the possibilities offered by the mass detector (*i.e.*, to separate and determine more than 40 components, in widely different concentrations, in the presence of each

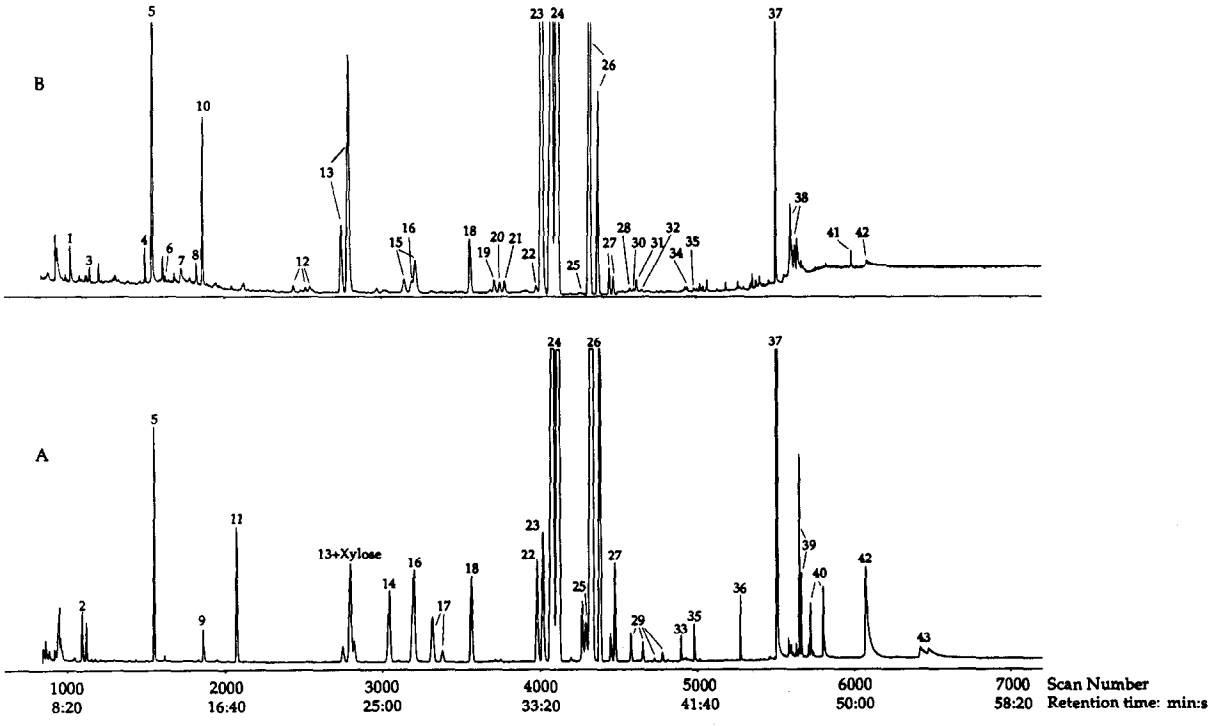

Fig. 1. Total ion chromatograms (TIC) of a (A) calibration solution and (B) an apple (Jonnee$_2$) sample. Peaks in order of retention times (min:s, in parentheses), 1 (8:35) = Phosphoric acid; 2 (9:07) = succinic acid; 3 (9:36) = pyruvic acid; 4 (12:32) = 5-hydroxy-N-valeric acid; 5 (12:55) = malic acid; 6 (13:36) = butanal; 7 (14:26) = 3-methyl-2-hydroxy-2-butenoic acid; 8 (15:13) = 1,2-hydroxycyclohexene; 9 (15:29) = pimelic acid; 10 (15:33) = 2-deoxy-D-erythrose; 11 (17:16) = tartaric acid; 12 (20:22, 20:59, 21:13) = xylitol; 13 (22:54, 23:16) = arabinose; 14 (25:22) = caffeic acid; 15 (26:13, 26:47) = D-ribose; 16 (26:36) = citric acid; 17 (27:37, 28:08) = rhamnose; 18 (29:41) = quinic acid; 19 (30:58) = D-*erythro*-tetrafuranose; 20 (31:14) = talose; 21 (31:29) = 2-ketogluconic acid; 22 (33:10) = mannitol; 23 (33:30) = sorbitol; 24 (34:03, 34:22) = fructose; 25 (35:35) = galactose; 26 (36:11, 36:33) = glucose; 27 (37:04, 37:19) = fructose (open form); 28 (38:09) = glucaric acid; 29 (38:10, 38:46, 39:22, 39:47) = galacturonic acid; 30 (38:30) = lactose; 31 (83:30) = *meso*-inositol; 32 (38:47) = gluconic acid; 33 (38:48) = linoleic acid; 34 (40:48) = glucuronic acid; 35 (41:31) = stearic acid; 36 (43:56) = arachidic acid; 37 (45:53) = sucrose; 38 (46:29, 46:36) = turanose; 39 (47:05, 47:11) = maltose; 40 (47:41, 48:21) = chlorogenic acid; 41 (49:53) = β-sitosterol; 42 (50:42) = raffinose; 43 (53:29, 53:59) = maltotriose.

Table 2
Reproducibility of the simultaneous determination of organic acids and sugars as TMS or TMS-oxime derivatives

No.	Compound	Integrator units equivalent to 1 ng of substance ($n = 3$)					
		A		B		C	
		Mean ± S.D.	R.S.D. (%)	Mean ± S.D.	R.S.D. (%)	Mean ± S.D.	R.S.D. (%)
2	Succinic acid	809 ± 19.1	2.3	862 ± 20.4	2.3	795 ± 49.0	6.1
5	Malic acid	2280 ± 40.7	1.8	2931 ± 44.5	1.5	3273 ± 133.7	4.1
9	Pimelic acid	1375 ± 26.6	1.9	1419 ± 35.6	2.5	1083 ± 115.0	11
11	Tartaric acid	4381 ± 55.7	1.3	5262 ± 109.8	2.1	3976 ± 225.3	5.7
13	Arabinose + xylose	5667 ± 102.8	1.8	6760 ± 129.9	1.9	6074[a]	
14	Caffeic acid	2204 ± 133.3	6.0	2504 ± 176.6	7.1	2641 ± 60.1	2.2
16	Citric acid	4691 ± 266.3	5.7	5051[a]		3831 ± 277.5	7.2
17	Rhamnose	2827 ± 27.7	1.0	5709 ± 40.4	0.7	5850 ± 94.1	1.6
18	Quinic acid	6898 ± 352.3	5.1	7844 ± 453.0	5.8	7736 ± 309.7	4.0
22	Mannitol	7883 ± 404.0	5.1	9545[a]		9536 ± 236.8	2.5
23	Sorbitol	7711 ± 184.6	2.4	8717 ± 340.4	3.9	8962 ± 534.7	6.0
24	Fructose	7656 ± 153.6	2.0	8592 ± 398.6	4.6	9036 ± 528.9	5.9
26	Glucose	6946 ± 191.9	2.8	7863 ± 193.1	2.4	8426 ± 171.1	2.0
29	Galacturonic acid	1998 ± 56.5	2.8	1303 ± 35.0	2.7	1278 ± 53.4	4.2
33	Linoleic acid	349 ± 7.6	2.2	323 ± 10.4	3.2	223 ± 14.5	6.5
35	Stearic acid	825 ± 21.5	2.6	898 ± 10.8	1.2	784 ± 47.6	6.0
36	Arachidic acid	760 ± 9.5	1.3	746 ± 18.1	2.4	506 ± 31.0	6.1
37	Sucrose	6439 ± 235.7	3.7	7284 ± 173.4	2.4	7443 ± 247.4	3.3
39	Maltose	4754 ± 166.8	3.5	5309 ± 166.9	3.1	5263 ± 186.4	3.5
40	Chlorogenic acid	857 ± 15.5	1.8	997 ± 30.0	3.0	–	–
42	Raffinose	1863 ± 58.9	3.2	2007 ± 46.1	2.3	–	–

Amounts injected: (A) 20 ng of the minor constituents and 250 ng of the main constituents (fructose, glucose, sucrose); (B) 8 ng of the minor constituents and 100 ng of the main constituents; (C) 4 ng of the minor constituents and 50 ng of the main constituents. S.D. = standard deviation; R.S.D. = relative standard deviation. Numbers in the first column refer to the peaks in Fig. 1A.
[a] $n = 2$.

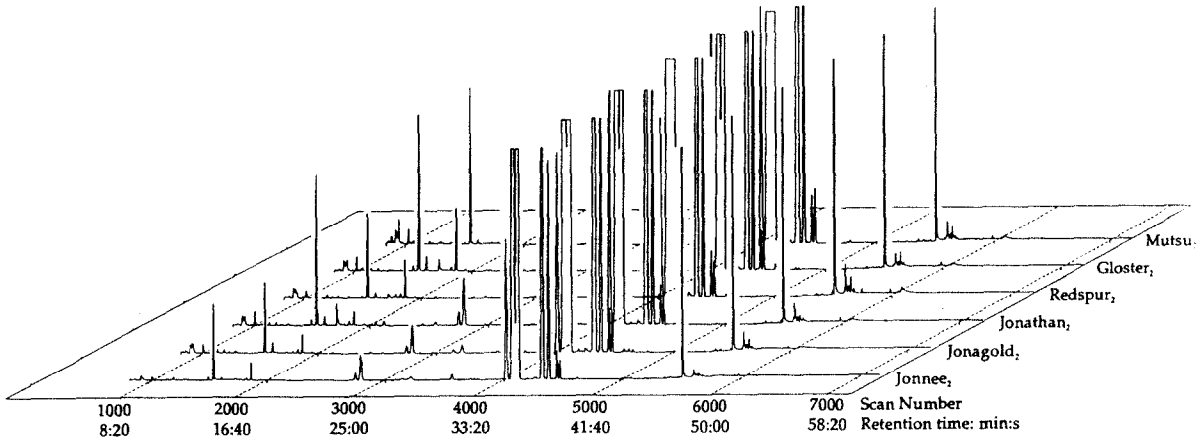

Fig. 2. TIC of the six apple varieties ($Jonnee_2$, $Jonagold_2$, $Jonathan_2$, $Redspur_2$, $Gloster_2$, $Mutsu_2$) presented in three dimensions. For further details, see Table 3).

other), the main task was to find the optimum conditions. In our earlier studies [2–7], a CP-Sil 5CB column, coated with methylsilicone, showed an excellent performance in the separation of more than 30 TMS derivatives (including members of various series of carboxylic acids and sugars of different degrees of polymerization, but excluding similar components, such as aldonic, uronic or sugar dicarboxylic acids). A 30-m DB-5 column, coated with 5% phenyl- and 95% methylsilicone, proved to be a good solution of the determination of all characteristic components of apples, similarly to the separation of citric acid from isocitric acid present in lemon and grapefruit samples [8].

The quantitative evaluation of the components were performed on the basis of the total ion count (TIC) data applying external standards (Table 2, Fig. 1A). As can be seen (Table 2) the concentration proportionality of the compounds (expressed in integration units per 1 ng of substance injected), provided by the ITD, was good, but not completely linear. This experience is in accordance with literature data [11]. Therefore,

Table 3
Compositions of six different apple varieties, expressed as percentages (w/w) of their dry matter contents (1992, B tests)

No.[a]	Component	$Jonnee_2$	$Jonagold_2$	$Jonathan_2$	$Redspur_2$	$Gloster_2$	$Mutsu_2$
	Dry matter content (%)	12.14	11.51	12.27	13.04	12.76	11.19
1	Phosphoric acid	0.10	0.21	0.29	0.16	0.30	0.35
3	Pyruvic acid × 10^2	3.6	7.5	6.2	6.7	6.5	4.1
4	5-Hydroxy-N-valeric acid × 10^2	8.2	–	–	–	–	3.3
5	Malic acid	1.8	1.4	3.2	1.6	3.3	3.8
6	Butanal × 10^2	1.5	2.2	2.9	–	–	2.8
7	3-Methyl-2-hydroxy-2-butenoic acid × 10^2	7.3	4.1	57.0	–	2.7	1.4
8	1,2-Hydroxy-cyclohexene × 10^2	6.7	11.0	11.0	11.0	10.0	6.5
10	2-Deoxy-D-erythrose	0.19	0.19	0.13	0.36	0.59	0.18
12	Xylitol × 10^2	3.6	5.5	14.0	4.4	6.1	9.5
13	Arabinose	0.79	0.67	1.29	1.31	0.47	1.50
15	D-Ribose × 10^2	7.2	30.0	20.0	36.0	14.0	–
16	Citric acid × 10^3	9.4	–	–	9.3	9.4	120.0
18	Quinic acid	0.11	0.22	0.19	0.15	0.35	0.29
19	D-*erythro*-Tetrafuranose × 10^2	5.2	10.0	11.0	8.9	12.0	3.8
20	Talose × 10^2	3.9	9.3	8.4	9.4	7.4	1.4
21	2-Ketogluconic acid × 10^2	1.9	1.1	1.5	9.7	7.8	9.1
22	Mannitol × 10^3	8.0	17.7	16.4	12.0	1.16	33.2
23	Sorbitol	2.0	3.0	3.5	2.7	4.0	4.0
24	Fructose	33.7	54.1	47.1	51.0	44.8	47.3
25	Galactose × 10^3	20.8	17.1	15.8	35.1	6.79	97.5
26	Glucose	12.8	22.4	19.4	15.6	20.6	27.9
27	Fructose (open form)	0.50	0.82	0.62	0.76	0.77	1.13
28	Glucaric acid × 10^2	1.2	12.0	2.1	8.9	19.0	27.0
30	Lactose × 10^3	10.2	32.3	6.2	15.5	16.7	41.5
31	*meso*-Inositol × 10^2	4.6	12.0	7.0	87.0	16.0	54.0
32	Gluconic acid × 10^2	1.1	9.6	–	6.8	14.0	19.0
34	Glucuronic acid × 10^2	1.7	4.9	–	1.6	3.5	0.6
35	Stearic acid × 10^2	3.3	2.2	–	2.8	3.5	3.9
37	Sucrose	6.2	11.0	9.1	15.4	10.4	9.6
38	Turanose × 10^2	17.0	25.0	18.0	31.0	18.0	2.4
41	β-Sitosterol × 10^2	4.3	4.6	6.8	8.1	7.5	1.4
42	Raffinose × 10^2	3.0	7.5	19.0	45.0	24.0	17.0
	Identified in total w/w%	59.1	95.7	86.7	91.9	87.3	98.0

[a] Numbers refer to the peaks in Fig. 1.

Table 4
Variations in the amounts of the main components of six apple varieties as a function of the times of their gathering (Jonnee$_{1-3}$, Mutsu$_{1-3}$) and of their storage (O, A, B, C), expressed as percentages (w/w) of their dry matter contents

Component		Jonnee$_1$	Jonnee$_2$	Jonnee$_3$	Jonagold$_1$	Jonagold$_2$	Jonagold$_3$	Jonathan$_1$	Jonathan$_2$	Jonathan$_3$	Redspur$_1$	Redspur$_2$	Redspur$_3$	Gloster$_1$	Gloster$_2$	Gloster$_3$	Mutsu$_1$	Mutsu$_2$	Mustu$_3$
Malic acid	O	5.1	4.6	3.9	4.2	5.7	4.3	6.4	6.2	4.8	3.4	1.8	2.1	6.2	5.2	5.4	6.6	5.6	5.3
	A	1.7	2.2	2.6	2.7	2.2	1.5	2.8	3.0	3.9	2.0	1.0	0.8	2.7	2.6	2.3	2.7	2.0	2.0
	B	2.1	1.6	1.6	1.7	1.7	1.6	2.2	2.3	2.3	0.9	1.2	1.3	2.6	2.5	2.6	1.9	2.0	1.9
	C	2.7	1.4	1.6	2.4	1.8	1.4	2.7	2.5	2.5	1.3	1.1	1.0	2.4	2.6	3.0	1.8	2.1	2.1
Arabinose	O	0.5	0.4	0.4	0.4	0.5	0.5	0.4	0.6	0.5	0.6	0.7	0.6	0.3	0.3	0.3	0.5	0.4	0.4
	A	0.5	0.5	0.4	0.2	0.2	0.2	0.4	0.4	0.4	0.7	0.5	0.1	0.3	0.2	0.2	0.4	0.4	0.4
	B	0.5	0.2	0.2	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.4	0.5	0.4
	C	0.5	0.2	0.2	0.3	0.3	0.2	0.3	0.3	0.3	0.4	0.3	0.3	0.2	0.2	0.2	0.3	0.2	0.3
Sorbit	O	2.0	2.3	2.4	2.1	1.9	2.1	1.7	1.1	1.1	1.7	1.0	1.1	2.8	2.1	2.7	2.3	1.9	2.2
	A	1.3	1.3	1.4	1.6	1.9	1.4	1.3	2.0	2.0	1.2	0.7	1.0	2.2	1.4	1.2	1.6	1.5	1.3
	B	1.9	1.3	1.3	1.5	1.3	1.8	1.6	1.5	1.7	0.5	0.5	1.0	1.3	1.2	1.5	1.6	1.9	1.2
	C	1.7	1.1	1.5	1.2	1.3	1.3	1.4	2.1	2.2	0.9	0.9	1.2	3.0	1.3	1.3	1.3	1.3	1.3
Fructose	O	42.9	39.3	41.5	42.2	41.0	51.0	42.2	40.4	52.6	44.8	44.7	48.0	36.9	37.3	39.3	43.4	36.3	43.5
	A	24.9	24.8	24.8	29.2	35.8	26.2	25.1	28.0	32.1	31.3	26.8	26.2	24.3	25.4	26.4	31.0	31.1	28.9
	B	29.5	29.2	28.8	24.7	23.0	22.2	24.6	25.5	22.7	18.4	27.0	19.6	20.7	22.3	22.7	25.6	27.0	28.4
	C	27.7	24.8	24.0	22.9	23.2	20.6	23.7	25.2	11.9	18.6	18.5	19.7	22.8	17.5	18.2	16.5	16.8	23.0
Glucose	O	8.1	7.6	11.0	12.3	7.7	17.7	7.7	8.2	15.8	18.1	17.7	19.1	9.9	16.6	13.8	8.2	6.0	9.8
	A	6.9	7.3	6.2	6.7	8.6	4.4	6.9	8.7	10.3	13.0	10.2	12.5	9.1	9.7	9.8	6.0	6.9	7.0
	B	8.2	8.3	7.4	7.0	4.3	5.9	7.0	7.6	6.8	7.9	9.9	7.4	9.9	9.6	9.5	8.2	8.2	7.2
	C	8.0	7.1	6.9	5.9	4.8	5.4	6.9	6.3	4.0	7.3	7.6	8.2	7.8	7.2	7.9	4.4	6.0	8.1
Sucrose	O	22.2	22.1	24.1	25.8	34.2	22.6	26.0	26.7	35.6	12.4	13.4	11.1	23.2	24.2	3.10	23.8	28.9	31.5
	A	5.9	6.5	9.5	15.9	16.3	14.0	8.1	14.7	10.9	1.9	2.4	2.6	15.7	8.4	4.5	17.8	9.6	10.3
	B	8.9	8.9	9.8	8.5	13.2	13.0	10.0	7.6	7.8	1.8	3.0	2.2	4.9	7.7	8.9	3.8	6.1	4.1
	C	5.0	5.4	6.3	9.9	13.3	9.5	5.3	8.5	10.8	1.6	1.5	1.5	11.8	6.0	5.7	5.4	2.3	1.6

to obtain acceptable and reliable analytical values for the calculation of the composition of apple samples (Tables 3 and 4), the corresponding responses (Table 2) were taken into account.

In the determination of those components that were not present in the external standard solution, the closest eluting member of standard solution, *i.e.*, the corresponding carboxylic acid or sugar (depending on the compound to be determined), served as the basis of calculation.

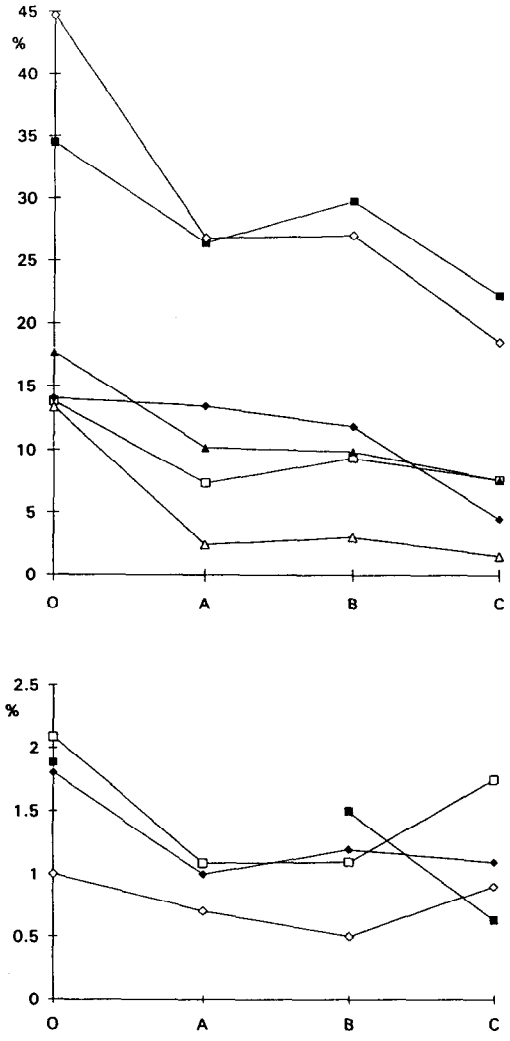

Fig. 3. Changes in the amounts of the main components, measured in varieties Redspur$_2$ (B tests), obtained in two consecutive years. The ordinates represent the percentages of components expressed as the corresponding dry matter content of the samples. For O, A, B and C, see Experimental) Top: ■ = fructose (1992); □ = glucose (1992); ◆ = sucrose (1992); ◇ = fructose (1991); ▲ = glucose (1991); △ = sucrose (1991). Bottom: ■ = malic acid (1992); □ = sorbitol (1992); ◆ = malic acid (1991); ◇ = sorbitol (1991).

For the identification of those apple constituents which were not available in our standard solution, selected ion monitoring was applied, utilizing the mass selectivity of the fragments of silylated compounds, provided by the characteristic electron impact (EI) mass spectra, present in the NIST or Wiley libraries, mostly in both (Fig. 1B, Table 3).

Evaluating the composition of various apple varieties, gathered and stored under the same conditions (Fig. 2, Table 3), it is obvious that considerable differences were measured (Fig. 2), mainly in the concentrations of the minor constituents (Table 3). High levels of 3-methyl-2-hydroxy-2-butenoic acid and xylitol (Jonathan), citric acid, mannitol, galactose, glucaric acid, lactose and gluconic acid (Mutsu) and D-ribose, *meso*-inositol, turanose and raffinose (Redspur) were found. The distribution also of the main constituents proved to be characteristic of the variety.

Variations in the amounts of the main constituents, due to the date of gathering and storage times, are compiled in Table 4 and Fig. 3.

It can be stated, both on the basis of values obtained from samples collected in 1991 (data in Table 3) and in 1992 (data in Fig. 3) and in comparison with each other, that the amounts of the main constituents decrease with increasing storage time. Large losses on storage were found in the fructose and sucrose contents of all varieties, independently of their date of gathering.

4. Conclusions

A CS–MS method was developed for the simultaneous determination of sugars and acids, including aldonic-, uronic- and sugar dicarboxylic acids, as their TMS oxime and TMS derivatives, present in six different apple varieties. The determination of the components was based on

the evaluation of their TIC. The identification and determination of those compounds which were not available was based on their EI mass spectra provided by the NIST and/or Wiley libraries.

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