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Simultaneous Identification and Quantification of the Sugar, Sugar Alcohol, and Carboxylic Acid Contents of Sour Cherry, Apple, and Ber Fruits, as Their Trimethylsilyl Derivatives, by Gas Chromatography−Mass Spectrometry

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Our gas chromatography−mass spectrometry method—developed for the simultaneous quantitation of mono-, di-, and trisaccharides, sugar alcohols, caboxylic and amino acids, measured as their trimethylsilyl-(oxime) ether/ester derivatives, from one solution by a single injection, prepared in the presence of the fruit matrix—has been extended/utilized for special purposes. The compositions of (i) freshly harvested and stored sour cherries (*Prunus cerasus*), (ii) apples obtained from organic and integrated productions (*Malus domestica*), and (iii) green and ripe beers (*Zizyphus mauritiana* L.) were compared. On the basis of earlier, basic researches (derivatization, quantitation, and fragmentation studies of authentic compounds), we demonstrate the reproducible quantitation of the main and minor constituents in a wide concentration range ($\sim 1 \times 10^{-3}$ to $\geq 40\%$, in total up to $\leq 98\%$, calculated on dry matter basis of the fruit matrices). Reproducibility of quantitations, calculated on the basis of their total ion current values, provided an average reproducibility of 3.3 (sour cherries), 6.2 (apple), and 4.3 (ber) RSD %, respectively.

KEYWORDS: Sour cherry; organic/integrated apple; ber; GC-MS identification/quantitation; silyl derivatives; sugars; sugar alcohols; carboxylic/amino acids

INTRODUCTION

The need for the simultaneous analysis of acids and sugars is associated with the fact that a number of natural matrices (fruits, vegetables, drug and industrial plants, mushrooms, honeys, ferment liquors, etc.) do consist in overwhelming part (∼70−100%) of these groups of organic compounds. The knowledge of the quality and quantity of sugars and acids, as well as the ratios of the single saccharides to each other and the ratios of the sugars to the acids, proved to be in particular important from several points of view: providing information on the general quality, freshness, maturity, storability, and/or the optimization of selected technological processes. Thus, in all of those laboratories, which are specialized in the analysis of the above detailed matrices, at least some of the total acid or total sugar determinations are to be performed. Unfortunately, these so-called total determination processes, almost without exception, are providing misleading results.

The development of a wide variety of chromatographic methods including the group of sugars, sugar alcohols, and carboxylic and amino acids furnishes up-to-date possibilities to solve this analytical task by means of chromatography, mainly by gas chromatography (GC) and high-performance liquid chromatography (HPLC). The advantage/disadvantage phenomena of the two main chromatographic techniques reflecting the view of an analytical chemist, deeply involved also in food analysis, having access to both systems (GC and HPLC), have been discussed in detail (*1*). Chromatographic proposals published for the quantitation of the sugar and carboxylic and amino acid contents of food matrices were reviewed recently (*2*). The analysis system of the simultaneous quantitation of sugars and acids was developed continuously in our laboratory, without any preliminary isolation process, in the presence of the matrix and was published from 1990 on, by studying, optimizing, and thereafter applying the technique for various matrices.

Basic research studies were associated with the topics as follows: (i) Studies were associated with the single step silylation process of acids, sugars, and sugar alcohols (*3*). This procedure furnished limited possibilities, because of the poor solubility of the reagent. (ii) Studies were associated with the recognition of the intrinsic feature of the oximation/silylation of reducing sugars. These investigations resulted in the unambiguous and reproducible quantitation of reducing sugars (*4*, *5*) providing a powerful tool in the analysis of mono- (pentoses, hexoses), di-, and trisaccharides and sugar alcohols (mannitol,

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Table 1. Temperature Program of the Column and that of the Injector

Column

segment	temp	rate	time
1	60	0.0	2.0
2	120	20.0	3.0
3	155	6.0	5.83
4	155	0.0	10.00
5	250	13.0	7.30
6	250	0.0	12.0
7	330	20.0	4.0
8	330	0.0	10.0
	total	54.13	

Injector

segment	temp	rate	time
1	60	0.0	2.00
2	320	180.0	1.44
3	320	0.0	6.00

Table 2. Reproducibility in the Quantitation of Various Amounts of Acids, Sugars, and Sugar Alcohols from Model Solutions on the Basis of TIC Values[a]

	integrator units (ng)				
	injected amounts (ng/1 μL)				
compounds: A, B, C (ng)	A	B	C	average	RSD %
succinic acid A: 30.51	(694.0)	941.8	896.5	919.2	2.5
C10 carboxylic acid A: 7.22	478.1	455.4	453.8	462.4	2.4
malic acid A: 39.55	1141.8	1168.3	(1372.0)	1155.1	1.1
tartaric acid A: 26.69	1863.8	1899.2	1957.1	1906.7	2.0
citric acid A: 27.59	1235.7	1265.6	1393.0	1298.1	5.3
quinic acid A: 28.83	2648.7	2608.1	2675.7	2644.2	1.1
sorbitol A: 42.15	2219.5	2063.0	2326.6	2203.0	4.9
fructose A: 9.46	3231.1	2986.1	2862.4	3026.5	5.1
glucose A: 13.70	(2790.4)	2430.0	2377.6	2403.8	1.5
galacturonic acid A: 30.24	1572.9	1616.7	1658.9	1616.2	2.2
inositol A: 30.59	3292.6	3171.0	(3697.1)	3231.8	2.7
stearinic acid A: 6.86	655.3	661.2	581.6	632.7	7.0

[a] Indications: A ng = the maximum injected amounts; B = A/2; and C = A/4. Values in parentheses have been omitted from the mean.

sorbitol), present in enormous excess in comparison to acids. (iii) Fragmentation studies of different homologous series of aromatic and aliphatic hydroxy-, mono-, di-, and tricarboxylic, phenolic (*6*, *7*), and amino acids (*8*) as their trimethylsilyl (TMS)-(oxime) ether/ester derivatives made possible their identification and quantitation in extremely different concentrations, in enormous excess of sugars, not only on the basis of their total ion current (TIC) but also on that of their selective fragment ion (SFI) values.

Advantages of direct derivatization, in the presence of the natural matrix and in comparison to different extraction/isolation processes, were proved first, in the case of sugars only (*9*, *10*). Later on, it was confirmed also in the direct derivatization of sugars, sugar alcohols, and amino and carboxylic acids of apricots, simultaneously (*11*, *12*). Optimum derivatization and chromatographic conditions were described for apples (*13*, *14*), for different vegetables, including strawberries (*15*), for various citrus fruits, bananas, grapes, and pears (*3*, *16*, *17*), applying all methods up to the 1994 GC-flame ionization detection method. From 1994 on, the GC-MS procedure was performed for citrus fruits only (*18*), for high chlorogenic acid-containing potato extracts (*19*), for honeys (*5*, *20*), for several Basidiomicetes mushrooms (*21*), and recently also for sour cherry fruits (*22*).

The aim of this paper is to show the simplicity/effectiveness and usefulness of the above detailed chromatographic technique and to compare the sugar, sugar alcohol, and carboxylic and amino acid contents of (i) three freshly gathered and stored sour cherry cultivars, (ii) four freshly gathered and stored apple cultivars obtained from organic and integrated productions, and (iii) two ber cultivars determined in unripe and ripe conditions.

EXPERIMENTAL PROCEDURES

Materials and reagents were of analytical reagent grade. Model sugars, sugar alcohols, carboxylic and amino acids, as well as pyridine and hydroxylamine hydrochloride were from Reanal (Budapest, Hungary); hexamethyldisilazane (HMDS) was from Fluka (Buchs, Switzerland); and trifluoroacetic acid (TFA) was from Serva (Heidelberg, Germany).

Table 3. Sugar/Sugar Alcohol and Carboxylic Acid Composition of Three Sour Cherries, Determined as Their TMS–(Oxime) Ether/Ester Derivatives by GC-MS[a]

		compound %, expressed in percenetages of their dry matter contents					
		sour cherry varieties					
		Érdi bötermö		Pándy		Kántorjánosi	
		E0	E1	P0	P1	K0	K1
		dry matter content (%)					
compound	retention time (min)	13.9	14.9	16.8	15.8	16.8	17.4
glycolic acid		1.67	0.70 (2.13)	2.39	0.68 (1.1)	1.69	0.35 (4.0)
phosphoric acid	7.46	0.65 (4.4)	0.51 (4.3)	0.82 (0.16)	0.58(1.0)	0.79 (5.2)	0.49 (5.5)
malic acid	11.21	9.1 (1.8)	6.4 (1.8)	11.6 (7.6)	9.7 (3.4)	9.13 (1.5)	8.8 (2.4)
xylose	19.31/19.53	0.087 (1.8)	0.084 (1.0)	0.12 (0.9)	0.093 (4.3)	0.087 (1.3)	0.079 (4.6)
rhamnose	22.45/22.56	0.019 (13)		0.013 (2.3)			
citric acid	23.04	0.057 (9.2)	0.10 (2.9)	0.12 (5.0)	0.052 (0.058)	0.12 (14.9)	0.061 (7.0)
quinic acid	23.38	0.070 (1.6)	0.059 (7.4)	0.13 (2.1)	0.081 (2.5)	0.11 (2.7)	0.080 (2.6)
sorbitol	24.56	10.8 (5.7)	9.8 (2.3)	18.5 (1.8)	18.5 (0.71)	14.3 (0.25)	14.3 (1.6)
fructose	25.05/25.13	31.3 (1.1)	39.7 (2.9)	28.1 (1.3)	32.9 (2.8)	26.5 (2.8)	29.2 (0.4)
glucose	25.55/26.05	36.9 (4.3)	31.9 (1.5)	37.8 (4.1)	34.3 (4.2)	32.6 (1.2)	29.2(1.5)
inositol	26.58	0.11 (3.5)	0.089 (4.8)	0.07 (3.3)	0.052 (5.5)	0.05 (4.3)	0.037 (7.2)
stearinic acid	28.56	0.090 (0.87)	0.089 (2.5)	0.085 (2.7)	0.069 (10)	0.076 (0.7)	0.049 (2.0)
sucrose	33.17	0.035 (3.5)	0.030 (8.9)	0.023 (7.6)	0.021 (4.4)	0.031 (1.0)	0.019 (2.4)
disaccharides	35.20–41.30	0.46 (0.51)	0.24 (1.8)	0.39(1.0)	0.33 (0.35)	0.34 (2.4)	0.34 (1.9)
raffinose	44.94	0.12 (4.3)	0.099 (1.2)	0.17 (3.5)	0.13 (6.3)	0.15 (0.0)	0.10 (4.9)
identified in total		91.5	89.8	100.3	97.5	86.0	83.1

[a] Indications as in **Table 2**, as well as values in parentheses, represent the averages of at least three separate derivatizations; blank entry = below the detection limit.

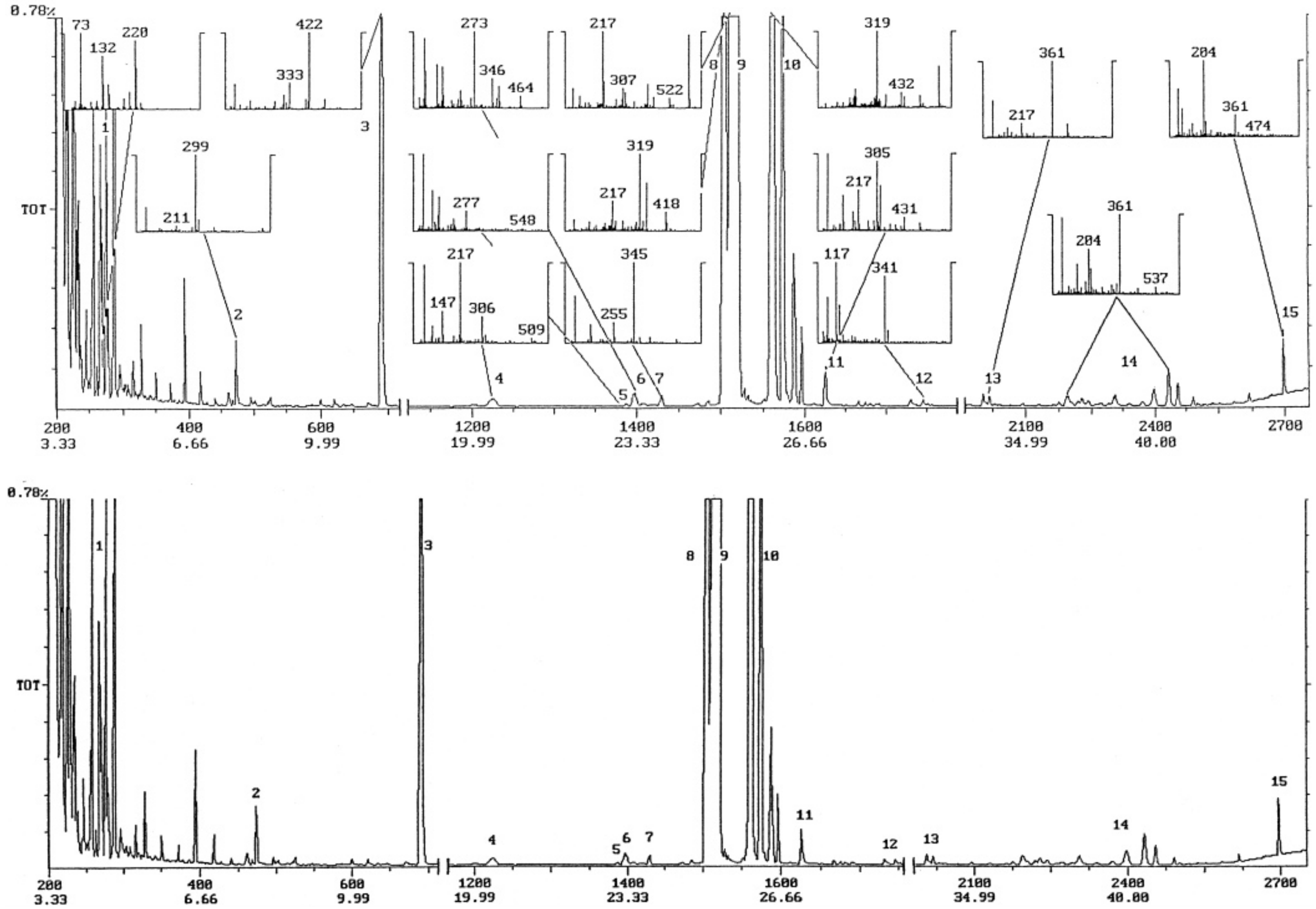

Figure 1. TIC chromatograms and the corresponding fragments of the TMS–(oxime) ether/ester derivatives obtained from the sour cherry cultivar Érdi Bötermö, E0. Peaks: 1, glycolic acid; 2, phosphoric acid; 3, malic acid; 4, xylose; 5, rhamnose; 6, citric acid; 7, quinic acid; 8, sorbitol; 9, fructose; 10, glucose; 11, inositol; 12, stearinic acid; 13, sucrose; and 14, disaccharides (not identified) in the range of 35:20–41:30 min retention time (detailed data in **Table 3**). The *y*- and *x*-axes of the inserts are as follows: *y* = relative intensity of masses (*m*/*z*); *x* = masses (*m*/*z*) extracted from the apex of selected peaks.

Authentic sour cherry (*Prunus cerasus*) cultivars (cv.), such as Érdi Bőtermő (E), Kántorjánosi (K), and Pándy (P), were obtained immediately from the Fruit Research Institute (Érd, Elviramajor, Hungary). Freshly harvested samples at various ripening times (E0, 17.06.02; K0, 25.06.02; and P0, 27.06.02), as well as samples after storage in the Central Institute of Food Research, at 4−6 °C, for different periods of time (E1, 01.07.02; K1, 08.07.02; and P1, 08.07.02), were indicated by the 0 (E0, P0, and K0) and by the 1 (E1, K1, and P1) indices, respectively. (Samples from the years of 2001 and 2002 were marked accordingly.)

Washed (thereafter by paper wiped to dryness), seedless 100 g fruits, measured with 0.05 g precision, were put into a potato-masher previously covered by 3-fold gauze. The fruits were hand-pressed until juice and/or pulp was obtained (50−60 g, weighed with 0.05 g precision). The pressed juice/pulp was stored in the freezer in a ground stopped glass vial (further on, fruit/sour cherry/apple/ber pulp or fruit/sour cherry/apple/ber sample). The dry matter contents of the fruit pulps were determined by drying in an oven, at 80 °C, to a constant mass (overnight).

Authentic apple (*Malus domestica*) cultivars, such as Idared, Liberty, Pilot, and Pinova, were obtained from the experimental orchard of Debrecen University, Centre of Agricultural Sciences, Department of Horticulture (Debrecen, Hungary). The apples were obtained, in parallel, from “organic” and from “integrated” productions (further information on organic and integrated vs Idared, Liberty, Pilot, and Pinova: The date of harvesting was September 2000, and they were stored until February 2001, if not otherwise stated). Authentic ber (*Zizyphus mauritiana* L.) cultivars such as Reshmi and Umran were gifts, harvested in India, at 2002, in the green stage and ripened for 3 days at room temperature.

Preparation of the TMS-Oxime and TMS Derivatives. Model solutions containing various amounts of major (0.5×10^{-4} to 5×10^{-3} g of malic acid, glucose, fructose, and sucrose) and minor components (5×10^{-6} to 2.5×10^{-4} g of amino acids, *o*-phosphoric acids, carboxylic acids, polyalcohols, and sugars, all shown in **Table 2**), as well as ∼0.15−0.25 g of sour cherry, apple, or ber pulps (containing approximately the corresponding amounts of constituents to be derivatized) were evaporated to dryness in a rotary evaporator at 50−60 °C. The dehydrated residues were treated with 500 μL of pyridine (containing 2.5 g of hydroxylamine hydrochloride/100 mL) and were heated for 30 min at 70 °C. The cooled samples were then trimethylsilylated with 900 μL of HMDS and 100 μL of TFA in 4 mL Reacti vials for 60 min at 100 °C. Thereafter, the solutions were ready for the analysis. Depending on their sugar/acid contents, they were diluted with HMDS before analysis. The amounts of stock solutions injected into the GC-MS system were 1 μL of the derivatized stock solutions.

Separation of the TMS-(Oxime) Ether/Ester Derivatives. The apparatus was the SATURN II GC-MS system of Varian (Walnut Creek, CA), supplied with an ion trap detector (ITD), a Varian 8200 AutoSampler, and a septum-equipped programmable injector (SPI). The column used was the product of J&W Scientific (Folsom, CA) (I.D. 2660081; DB-5, 30 m × 0.248 mm; df = 0.25 μm). The temperature program of both the column and the SPI proved to be the “reproducible optimum”, as shown in **Table 1**.

The temperature of the transfer line was 280 °C. The actual parameters of the ITD were defined by the automatic set up mode. Actual automatic setup conditions: mass range, 40−650 amu; seconds/

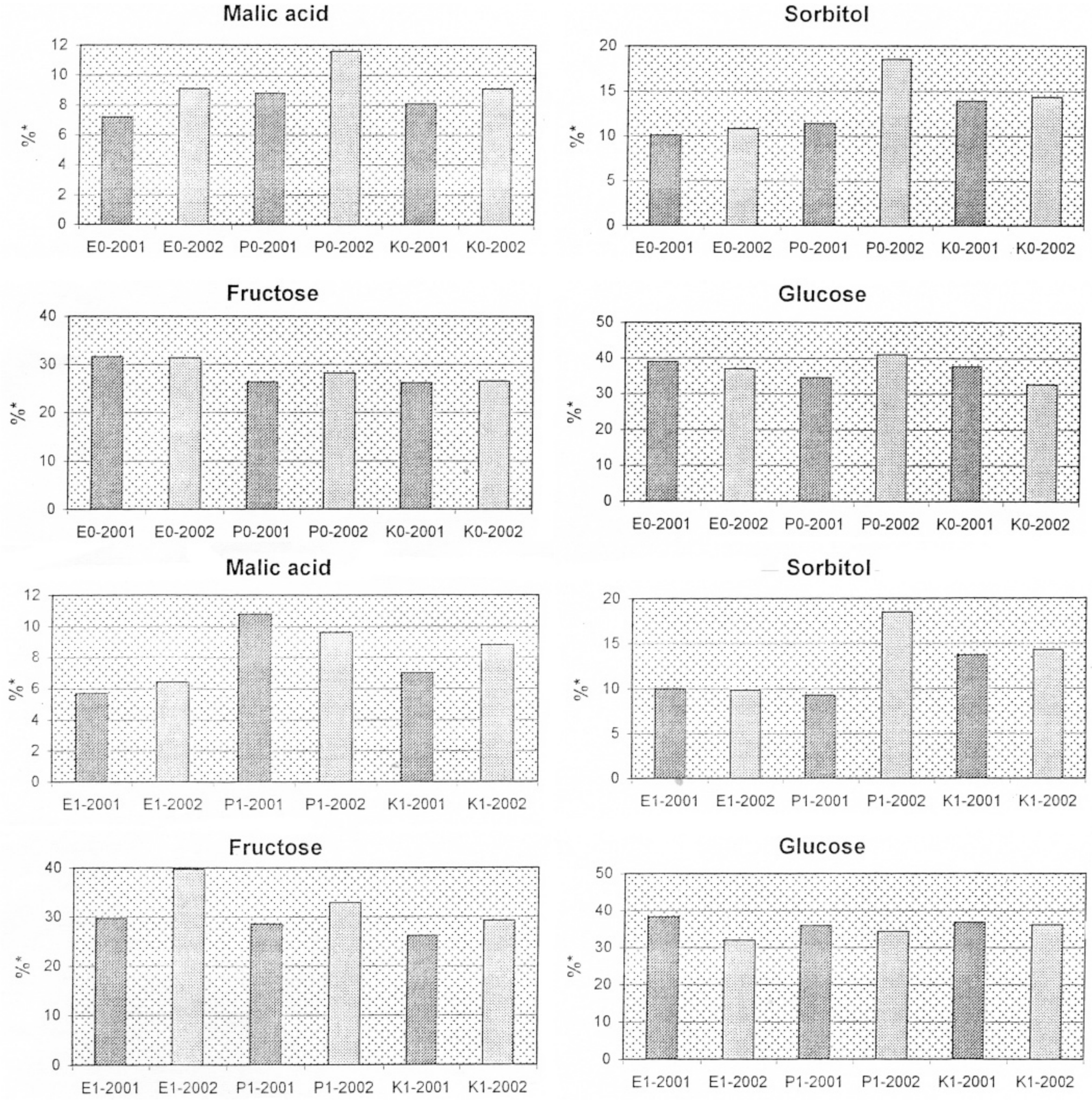

Figure 2. Changes of the malic acid, sorbitol, fructose, and glucose contents, determined as their TMS–(oxime) ether/ester derivatives, by GC-MS, in three sour cherry cultivars (Érdi Bötermö, E0; Pándy, P0; and Kántorjánosi, K0), immediately after gathering (panel **A** indicated as E0, P0, and K0) and after 2 weeks of storage (panel **B**, indicated as E1, P1, and K1) harvested in years 2001 and 2002, respectively.

scan, 1.000; acquire time, 54 min; Fil/Mul delay, 420 s; peak threshold 0 count; mass defect, 100 mmu/100 amu (atomic mass unit); and background mass, 50 amu.

RESULTS AND DISCUSSION

Studies on the Composition of Sour Cherries. *Literature Overview.* For the simultaneous analysis of the sugar, sugar alcohol, and carboxylic acid contents of sour cherries (*P. cerasus*), literature proposals have not been found. The sugar and sugar alcohol contents of the Montmorency cultivar were quantitated in its 80% ethyl alcoholic extracts by HPLC (*23*). The total of dissolved organics in sour cherries was measured by refractometry (*24*−*26*), and the total of acids was measured by titrimetry—even in 2003 (*26*), similarly, as two decades earlier (*23*). The aroma components were investigated in diethyl ether/pentane (2/1, v/v) extracts by GC-MS (*25*), while the total of carotenoids, subsequently to a long-lasting isolation/cleaning process, was investigated by spectrophotometry (*26*).

The overwhelming part of recent literature relates to the analysis of the flavonoid content of sour cherries. Partly on this basis, partly being encouraged on the general relevancy of this group of organics (*27*, *28*), and present in an overwhelming part of fruit matrices, we also decided to incorporate into our simultaneous analysis system the group of flavonoids (*22*).

Sugar, Sugar Alcohol, and Carboxylic and Amino Acids Contents of Sour Cherry Samples Determined as Their TMS-(Oxime) Ether/Ester Derivatives by GC-MS. GC-MS Studies in Model Solutions. GC-MS investigations performed in model

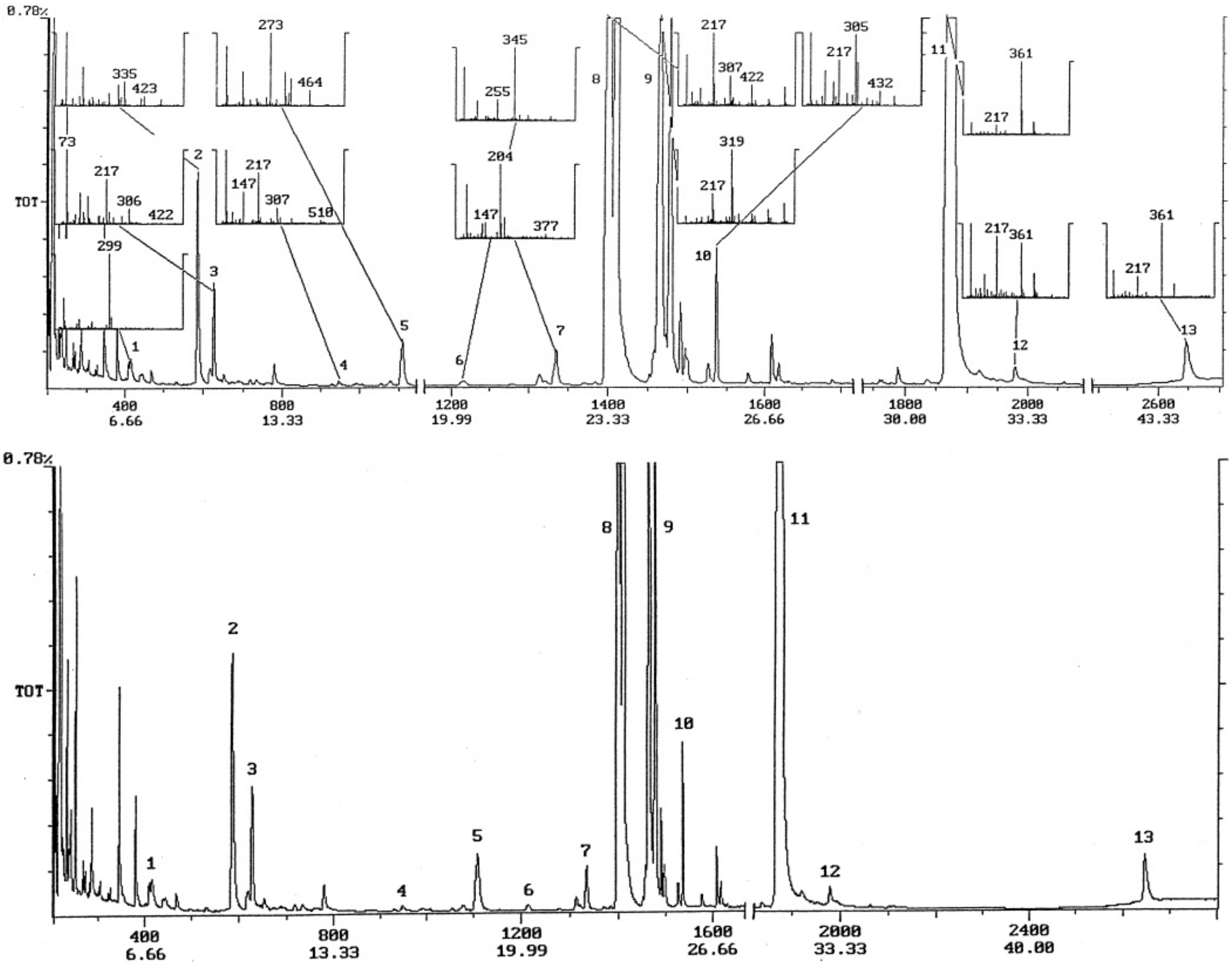

Figure 3. TIC chromatograms and the corresponding fragments of the TMS–(oxime) ether/ester derivatives obtained from the ber cultivar Reshmi green. Peaks: 1, phosphoric acid; 2, malic acid; 3, sugar acid type compounds; 4, xylose; 5, citric acid; 6, quinic acid; 7, pentose (not identified); 8, fructose; 9, glucose; 10, inositol; 11, sucrose; 12, disaccharide (not identified); and 13, raffinose (detailed data in **Table 6**). The *y*- and *x*-axes of the inserts are as follows: *y* = relative intensity of masses (*m*/*z*); *x* = masses (*m*/*z*) extracted from the apex of selected peaks.

solutions, with various expected compounds in sour cherries, including organic acids, *o*-phosphoric acids, and amino acids, in the presence of an enormous excess of saccharides of different degrees of polymerization (DP), yielded promising results. Preliminary tests proved that several acids, sugars, and sugar alcohols be assumed as major and minor constituents in sour cherries, identified on the basis of earlier fragmentation studies (*4*−*7*, *20*), and are present in extremely different concentrations. Unfortunately, amino acids could not be identified, very likely being present below their detection/quantitation limit (5/15ng/injection).

Introductory investigations carried out with three sour cherries (Érdi Bőtermő, E; Pándy, P; and Kántorjánosi, K) provided information concerning the type and quantity of constituents. On this basis, the minor and main constituents were determined in model solutions (**Table 2**). Major and minor constituents in the wide ranges of nanogram levels, in the presence of each other, were repeatedly identified on the basis of their SFI values and determined by means of TIC values. The only exception, covered by reagent peaks, was glycolic acid, which had to be quantitated on the basis of its SFI values (*6*, *7*), such as $[M]^{+\cdot} = m/z = 220$, $[M - CH_4]^{+\cdot} = m/z = 204$, and $[M - Si(CH_3)_4]^{+} = m/z = 132$ (**Table 3** and **Figure 1**). The quantitation of constituents on the basis of their TIC values (**Table 3**) proved to be acceptable (average RSD % = 3.3).

Quantitation of the Constituents of Sour Cherries as a Function of Cultivars and Storage Times: Year of Harvesting 2002. The amounts of various minor and major constituents of sour cherries harvested in the year of 2002, measured immediately (E0-2002, P0-2002, and K0-2002) and stored for 2 weeks (E1-2002, P1-2002, and K1-2002), are detailed in **Table 3** and in **Figure 1**. The main constituents, calculated on the basis of their TIC values, provided an average reproducibility of 2.5 RSD % (for components present in concentrations of ≥1%) and 3.6 RSD % (for components present in concentrations of ≤1%). The evaluation of the TMS sugar oximes was based on the fact that the ratios of their syn and anti forms are stable, independent of their amounts analyzed and characteristic only to the sugar species to be measured (*4*, *5*). As to the composition of the three sour cherry cultivars, considerable differences have not been found.

Regarding the impact of storage at 4 °C for 2 weeks (**Table 3**: comparing values for the freshly harvested samples E0, P0, and K0 to the corresponding stored ones E1, P1, and K1), it can be stated that in the case of all three cultivars (i) malic acid contents decreased considerably, while citric and quinic acid

Table 4. Composition of Four Apple Varieties, Measured Immediately after Gathering from Organic and Integrated Productions, Determined as Their TMS–(Oxime) Ether/Ester Derivatives by GC-MS[a]

		compound %, expressed in percentages of dry matter content of samples							
		apple varieties							
		Idared		Liberty		Pilot		Pinova	
		organic	integrated	organic	integrated	organic	integrated	organic	integrated
		dry matter content (%)							
compound	retention time (min)	14.4	12.2	18.3	14.6	15.6	14.6	15.9	14.1
phosphoric acid	6.43	0.080 (4.4)	0.11 (4.3)	0.21 (3.5)	0.098 (3.6)	0.099 (15)	0.081 (4.4)	0.15 (9.4)	0.24 (9.0)
malic acid	9.46	6.2 (6.8)	6.0 (3.5)	5.3 (4.0)	5.5 (3.9)	4.9 (1.7)	5.1 (8.3)	4.4 (6.2)	4.1 (3.1)
glutamic acid	10.09							0.17 (20)	0.19 (15)
alanine	11:31	0.22	0.30	0.23	0.31	0.072	0.086	0.010	0.024
xylose	15.43/15.57	0.089 (4.4)	0.14 (5.2)	0.15 (4.9)	0.22 (3.3)	0.16 (4.6)	0.17 (4.3)	0.14 (5.2)	0.14 (4.6)
citric acid	18.35	0.086 (13)	0.37	0.17 (2.3)	0.10	0.11	0.12	0.058	0.11
quinic acid	20.31			0.033 (4.3)	0.067 (13)	0.11 (8.0)	0.12 (11)	0.066	0.085
sorbitol	23.16	2.0 (5.3)	1.15 (1.9)	4.23 (0.20)	2.84 (9.2)	3.50 (0.10)	2.51 (4.2)	3.43 (0.6)	2.4 (2.6)
fructose	23.33/23.43	33.8 (6.0)	42.6 (3.3)	51.1 (4.9)	55.3 (5.67)	40.7 (1.0)	38.0 (0.74)	44.6 (2.50)	44.0 (2.5)
galactose	24.18/24.32	0.045(15)	0.036 (20)	0.049 (2.0)	0.048 (1.5)	0.03 (13)6	0.030 (2.7)	0.037 (2.4)	0.043 (21)
glucose	24.32/24.44	7.1 (3.9)	8.5 (6.6)	11.3 (1.5)	12.1 (8.2)	9.2 (3.1)	6.5 (6.5)	8.3 (1.7)	12.1 (2.9)
galacturonic acid	25.04/25.26	0.011 (6.1)		0.0075	0.0070	0.0013	0.0042	0.0016)	0.0077
palmitic acid	25.13	0.029	0.045	0.012	0.013	0.037	0.035	0.011	0.013
inositol	25.40	0.065 (7.6)	0.064 (1.1)	0.054 (2.6)	0.076 (20)	0.054 (1.3)	0.060 (1.2)	0.051 (1.4)	0.043 (1.7)
sucrose	31.12	17.1 (2.9)	19.6 (2.8)	17.3 (0.8)	9.8 (2.8)	26.7 (6.3)	21.6 (2.3)	12.0 (1.7)	12.6 (3.4)
raffinose	44.08	0.066 (25)	0.068 (18)	0.072 (1.0)	0.029 (17)	0.14 (5.2)	0.23 (3.1)	0.059 (3.6)	0.018 (28)
identified in total		66.9	79.0	90.0	86.5	85.8	74.6	73.5	76.1

[a] Indications as in **Tables 2** and **3**, as well as amino acid values without RSD percentages, have been evaluated from a 5-fold dilution, only once.

Table 5. Composition of Four Apple Varieties, Measured after 5 Months of Storage from Organic and Integrated Productions, Determined as Their TMS–(Oxime) Ether/Ester Derivatives by GC-MS[a]

		compound %, expressed in percentages of dry matter content of samples							
		apple varieties							
		Idared		Liberty		Pilot		Pinova	
		organic	integrated	organic	integrated	organic	integrated	organic	integrated
		dry matter content (%)							
compound	retention time (min)	13.4	12.9	18.3	14.6	15.6	14.6	15.9	14.1
phosphoric acid	6.43	0.088 (4.4)	0.10(4.3)	0.090 (14)	0.11 (20)	0.16(4.5)	0.081 (10)	0.18 (7.8)	0.33 (2.2)
malic acid	9.46	3.80 (1.9)	4.10 (12)	2.91 (4.1)	4.10 (2.4)	2.78 (0.52)	3.77 (3.1)	2.27 (9.3)	2.85 (3.5)
glutamic acid	10.09					1.20	1.80	0.12	0.27 (15)
alanine	11:31	0.47		0.97	0.31	0.060	0.0906	0.019	0.10
xylose	15.43/15.57	0.28 (5.1)	0.42 (1.7)	0.83 (3.4)	0.99 (3.5)	0.36 (2.0)	0.45 (6.3)	0.32 (8.8)	0.86(9.1)
citric acid	18.35	0.05 (11)	0.10 (5.0)	0.17 (2.3)	0.14	0.19	0.16	0.12	0.29
quinic acid	20.31			0.036 (3.9)	0.056 (6.4)	0.13 (0.0)	0.12 (13)	0.041	0.18 (12)
sorbitol	23.16	1.40 (5.3)	1.95 (5.4)	4.56 (0.30)	4.34 (0.65)	3.61 (1.8)	3.33 (6.6)	3.92 (6.3)	2.60 (1.9)
fructose	23.33/23.43	39.5 (3.2)	45.1 (8.3)	59.0 (6.7)	62.6 (0.56)	44.7 (0.5)	39.4 (2.3)	45.2 (4.8)	52.6 (6.6)
galactose	24.18/24.32	0.061 (2.3)	0.062 (17)	0.053 (16)	0.051 (2.8)	0.037 (19)	0.038 (9.4)	0.059 (0.0)	0.050 (28)
glucose	24.32/24.44	11.8 (1.2)	8.51 (3.6)	17.7 (0.9)	18.0 (1.2)	14.2 (3.0)	8.4 (0.8)	11.4 (8.0)	14.9 (2.9)
galacturonic acid	25.04/25.26	0.023 (18)		0.0026 (2.8)	0.0040 (19)	0.012 (6.1)	0.0073	0.0038)	0.0038
palmitic acid	25.13	0.044	0.049	0.013	0.050	0.034	0.035	0.020	0.027
inositol	25.40	0.10 (8.2)	0.062 (3.4)	0.065 (1.1)	0.050 (6.1)	0.050 (4.3)	0.050 (7.0)	0.059 (1.2)	0.061 (9.2)
sucrose	31.12	12.1 (7.6)	16.1 (4.4)	6.9 (10)	4.28 (1.8)	23.9 (0.88)	18.9 (0.0)	9.6 (3.7)	8.7 (1.6)
raffinose	44.08	0.097 (0.7)	0.056 (20)	0.063 (6.7)	0.041 (5.2)	0.16 (0.0)	0.24 (7.4)	0.11 (12)	0.22 (23)
identified in total		69.8	76.6	93.4	95.1	91.6	76.9	73.4	84.0

[a] Indications as in **Tables 2**–**4**.

contents decreased only slightly. (ii) Increasing fructose contents can likely be attributed to the hydrolysis of fructose-containing polysaccharides (*29*). To understand the decrease, glucose contents need further study. The efficiency of the proposed method is shown by the total of the identified and quantitated compounds (**Table 3**) that proved to be between 82.7 and 99.9%, from one solution by a single injection, in the concentration range of 4 orders of magnitude.

Impact of the Year of Harvesting. Comparing the composition of freshly harvested fruits from years 2001 to 2002 (**Figure 2A**) on the basis of their main constituents, it turned out that (i) malic acid and sorbitol contents proved to be higher in year 2002, while (ii) glucose and fructose percentages did not differ within the experimental error of our measurements.

As to the stability of the main sour cherry constituents, after 2 weeks of storage, in the successive years of 2001 and 2002 (**Figure 2B**), changes proved to be on the same scale: associated with the fact that authentic fruits, originating from the same place, treated with the same expertness and conscientiousness, provided reproducible characteristics.

Studies on the Composition of Apples, Obtained from Organic and Integrated Productions. *Literature Overview.*

Table 6. Sugar/Sugar Alcohol and Carboxylic Acid Composition of Two Varieties of Green and Yellow Ber Fruits, Determined as Their TMS–(Oxime) Ether/Ester Derivatives by GC-MS[a]

			compound %, expressed in percentages of dry matter content of samples							
			Ber varieties							
			Reshmi yellow					Reshmi green	Umran yellow	Umran green
			1	2	3	average	RSD %			
			dry matter (%)							
components	identified by SFI (*m*/*z*)	retention time (min)	14.52					11.60	13.21	10.99
phosphoric acid	299, 314	6.46	0.55	0.64	0.61	0.61	3.44	0.51	0.82	0.51
malic acid	307, 335	9.44	0.51	0.59	0.52	0.52	1.12	2.94	1.42	1.85
sugar acid type compounds	117, 217, 306	10.26	2.18	2.22	2.03	2.20	2.61	0.78	1.56	0.47
	117, 231, 320, 421	10.44	0.61	0.77	0.70	0.75	5.0	0.00	0.34	0.00
	117, 231, 320, 421	10.57	0.51	0.50	0.48	0.50	1.63	0.00	0.32	0.07
	274, 375	13.02	0.04	0.11	0.05	0.10	17	0.24	0.35	0.47
xylose	307, 524	15.33/15.47	0.04	0.03	0.04	0.04	13	0.03	0.12	0.07
pentose, NI	219, 422	16.30/16.45	0.22	0.18	0.18	0.18	0.00	0.03	0.17	0.01
citric acid	273, 347, 375	18.30	0.08	0.10	0.08	0.10	6.1	0.29	0.09	0.08
quinic caid	345, 255	20.16	0.01	0.02	0.02	0.02	-	0.03	0.00	0.00
hexose, NI	204, 376	22.12	0.08	0.09	0.09	0.09	5.7	0.10	0.11	0.14
mannitol	319	22.54	0.86	0.85	0.86	0.87	2.36	0.01	6.9	1.02
fructose	204, 319	23.20/23.29	14.3	13.6	13.8	14.1	1.64	11.3	10.3	15.3
glucose	204, 307	24.25/24.38	13.1	12.6	12.9	13.0	1.22	8.56	9.9	12.6
inositol	305	25.37	0.23	0.23	0.17	0.23	2.57	0.20	0.54	0.42
sucrose	361	31.12	60.7	60.6	62.2	61.1	1.08	66.2	57.6	51.8
disacchar., NI	361	34.29/34.49	0.52	0.61	0.51	0.58	5.5	0.00	0.22	0.05
raffinose	361	44.06	0.73	0.87	0.76	0.74	3.54	0.25	1.14	0.63
identified in total			95.3	94.6	96.0	95.7		91.5	91.9	85.5

[a] Indications, as in **Tables 2**–**5**, as well as SFI; *a*,*b*,*c* = parallel derivatizations; NI = not identified; disacchar. = disaccharides.

There are, in the moment, irreconcilable opinions published in the columns of *Nature* (*30*, *31*) regarding the advantages/disadvantages of organic contra conventional and/or integrated apple productions. American scientists, on the basis of their self-experiences (*30*, *32*, *33*), declared the primary importance/superiority of the organic apple production in many aspects: (i) Concerning the three farming possibilities, they obtained similar apple yields. (ii) Comparing the organic and integrated systems to the conventional one, the first two furnished higher soil quality and lower negative environmental impact than the conventional one, and (iii) the organic system produced sweeter and less tart apples and higher profitability in comparison to the two others. In response to the above statements, hard critiques were reported (*31*, *34*). On one hand (*34*), the above detailed conclusions—drawn from the available results that regarded them to be slight and/or not justified by the methods used—were completely questioned. On the other hand (*31*), organic agriculture was regarded as an ideology only that is to be substituted by an agricultural pragmatism and flexibility, integrating the best of traditional farming with modern technology. To this debate, we try to contribute with our modest but exact analytical chemical results regarding the distribution of the main components of apples from organic and integrated production, in parallel.

Impact of the Growing Technology on the Sugar/Sugar Alcohol and Carboxylic and Amino Acid Contents of Four Apple Cultivars, Measured as Their TMS-(Oxime) Ether/Ester Derivatives: Immediately after Gathering and after 5 Months of Storage. The compositions of the freshly gathered and stored fruits are shown in **Tables 4** and **5**, respectively. As seen, (i) it is a general observation that the dry matter contents of fruits from organic production, without exception, are higher than their corresponding integrated counterparts. (ii) The amounts of any other constituents, taking into account the reproducibility of our measurements, do not differ significantly. (iii) The storability of fruits obtained from the two different productions after 5 months storage (**Table 5**) is providing similar characteristics: changes, relating to the decreasing malic acid and increasing fructose and glucose contents proved to be comparable.

It is to be noted that comparative data of firmness and β-galactosidase and polygalacturonase enzyme activities obtained exactly from the same fruits, from the same year—in parallel with organic and integrated produced fruits—served as the completing evidence (*35*) for our experiences: Consequently, in our understanding (based on all of these above detailed data), differences that could have been attributed to the different type of fruit productions have not been found.

Studies on the Composition of Green and Ripe Bers. *Literature Overview.* To our knowledge, there is no literature data on the acid and sugar/sugar alcohol contents of the ber fruits. However, several papers were found studying the triterpenoid of *Ziziphus xylopyrus* (*36*), the lectin (*37*) and cyclopeptide alkaloid (*38*) of *Z. mauritiana*, the flavonoids (*39*–*41*), the antisweet substances (*42*), the fatty acid composition of its lipid fraction (*43*), and the multipurpose utility (*44*, *45*) of *Ziziphus jujuba*.

Impact of the Ripeness (Unripe/Green, Ripe/Yellow) on the Sugar/Sugar Alcohol and Carboxylic Acid Contents of Two Ber Cultivars, Measured as Their TMS-(Oxime) Ether/Ester Derivatives. Introductory investigations were performed on the basis of earlier experiences. Identifications and quantifications were carried out on the well-known characteristics of sugars and fragmentation patterns of fruit acids (**Table 6**). In accordance with the extreme sweet taste of ber fruits, enormous high sucrose contents have been measured.

Selected ber components proved to be characteristic to the varietes and to their ripeness as well. (i) The malic acid content proved to be characteristic both to the varieties and to the ripeness of bers: Reshmi and Umran in green/yellow were 2.94/0.52% and 1.85/1.42%, respectively. (ii) Xylose and unidentified pentose contents, probably due to hydrolysis processes, were considerably higher in the yellow than in the corresponding green fruits; the total of pentoses (xylose + unidentified), in Reshmi and Umran in order of listing, were as follows (yellow/green, 0.04 + 0.18%/0.03 + 0.03%; 0.12 + 0.017%/0.07 + 0.01%). (iii) Raffinose seems to also be a hydrolysis product; raffinose contents are unambiguously higher in yellow than in green fruits (Reshmi and Umran yellow/green: 0.74/0.25 and 1.14/0.63%, respectively).

In conclusion, the utility of the principle of the simultaneous quantitation of sugars, sugar alcohols, and carboxylic and amino acids [measured as their TMS-(oxime) ether/ester derivatives by GC-MS] has been demonstrated by the identification and quantitation of these constituents in three types of fruits. Concentration changes of compounds have been followed as a funtion of the fruit varieties and growing and storage conditions. (i) Three sour cherry varieties have been investigated in 2 consecutive years, immediately after gathering and after 2 weeks of storage. The data obtained proved that in the composition of the three sour cherry cultivars, considerable differences have not been found and 2 weeks of storage resulted in a decrease of malic, citric, and quinic acids and in the increase of fructose contents. (ii) The determination of the sugar, sugar alcohol, and carboxylic and amino acids compositions of four apple varieties, obtained from organic and integrated production, might be a moderate contribution to solve contradictory understandings; namely, within the experimental error of our measurements, the identity of these fruit samples of organic and integrated productions could be unambiguously confirmed. (iii) The study of the sugar, sugar alcohol, and carboxylic and amino acids compositions of two unripe and ripe ber varieties showed that these fruits contain in particular high sugar (mainly sucrose) and low acid type components.

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