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Simultaneous determination of sugars, sugar alcohols, acids and amino acids in apricots by gas chromatography–mass spectrometry

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Abstract

Our GC–MS method for the simultaneous quantitation of mono- di- and trisaccharides, sugar alcohols and acids, measured as their trimethylsilyl-oxime ether/ester derivatives, prepared in presence of the fruit matrix, from one solution by one injection has been extended. The reproducible determination of apricot constituents was performed both on the basis of total ion current (TIC) and selective fragment ion (SFI) values, ensuring identification and quantitation in a wide concentration range (~$1 \cdot 10^{-3}$ to ≥40%), calculated on dry matter basis of the samples). The GC–MS procedure was utilized to prove the advantage of the direct derivatization process, in the presence of the fruit matrix, and to determine the changes in the sugar/sugar alcohol/carboxylic acid/amino acid composition of two apricot cultivars, as a function of their harvesting dates and storage conditions. Reproducibility of quantitations, characterized by their RSD values, proved to be, 3.6% (TIC) and 4.3% (SFI).

Keywords: Apricots; Fruits; Food analysis; Sugars; Sugar alcohols; Carboxylic acids; Amino acids

1. Introduction

It is well known and widely accepted that the knowledge of the qualitative and quantitative distribution of sugars and acids in fruits, vegetables, honeys and several other natural matrices are of primary importance for a number of reasons. These compounds are the main constituents of natural matrices and are involved in very important characteristics, such as maturity, ripeness, quality, authenticity, storage conditions, etc.

Only a few papers concerning the chromatography of the sugar and acid contents of apricots are available [1–10]. The particulars of these articles are summarized in Table 1.

There are three main, rate determining steps of analyses; extraction, derivatization and reproducibility studies. All are of primary importance and all still need to be studied and improved (Table 1) [1–10]. In respect of extraction prior to the analysis/derivatization (Table 1, third vertical column) a number of time consuming protocols have been suggested: separations by alcohols of various concentrations (80% methanol, applying reflux [1] or room temperature [5], 50% [9,10] and 80% ethanol [7]), acidified cold acetone [2] or 4% metaphosporic acid+quartz sand [4]. With two exceptions [9,10], acids [1–5] and sugars [6] have been determined in separate works [1–6], or in the same articles applying different types of time consuming, tedious and costly quantitation procedures [7,8]. Derivatizations were followed, after different pretreatments, either in extracts [1–7,9,10], or in the centrifuged, clarified fruit pulp [8].

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Table 1
Advances in the chromatographic analysis of sugars and acids present in apricots (literature data)[a]

[Ref.] date	Group, determined	Extraction procedure	Chromatography, column, derivatives	RSD (%)	Compounds, found
Acids, only					
[1] 1983	Hydroxycinnamic acids	100 g/2×800 ml Met reflux under N_2 for 30 min, SPE elution by Met. Ac.a (0.5%)	Capillary GC–FID, SE 30-glass capillary 37 m×0.27 mm, Silyl; HPLC, LiChrosorb RP-18, 250×4 mm, (5 μm), UV detection	–	7 Quinic acid esters of caffeic, *p*-coumaric and ferulic acids
[2] 1991	Aromatic acids	20 g/20 ml cold acetone containing 0.5 ml conc. HCl, 10 min centrifugation, extraction 2×20 ml EtAc	Paper chromatography, Whatman No. 1 or 3MM; developed by *n*-butanol–acteic acid–water (40:10:22, v/v), UV fluorescence with and without NH_3 or chromogenic spray (diazotised sulfanilic acid)	–	Chlorogenic, caffeic and *p*-coumaric acids
[3] 1992	Benzoic, cinnamic acids	100 ml juice evaporated to 25 ml extraction: (1) 2x 15 ml ether+(2) 2×15 ml EtAc	HPLC, UV (PDA) detection, C_{18} Nov-Pak, 300×3.9 mm	–	5 Hydroxybenzoic, 6 hydoxycinnamic acids
[4] 1994	Aliphatic acids/vitamin C	20 g/50 ml HPO_3 (4%)+quartz sand (1 h shaken), filtered	HPLC, Chromsil C_{18} (no more data available) UV detection	–	Malic and citric acids
[5] 1994	Chlorogenic acids	200 g/400 ml 80% Met, 18 h (room temp), evaporation+butanol, Amberlite XAD-2	HPLC, LiChrochart 100 RP-18, 125×4 mm, 5 μm) UV (PDA) detection	±6	Chlorogenic acid+2 of its glycosides
Sugars, only					
[6] 1994	Sugars, sorbitol	–	HPLC, Polispher CHCA (Merck), 300×65 mm RI detection	–	Sucrose, glucose, fructose, sorbitol
Acids and sugars, separately					
[7] 1989	Sugars, fatty acids	Free sugars: 80% EtOH; fatty acids: ether (no more data)	Free sugars (Dubois et al., 1956); Fatty acids: as methyl esters by GC (AOAC, 1970)	–	Glucose, fructose, sucrose, xylose, C_8–C_{18} ($C_{18:1}$–$C_{18:3}$) fatty acids
[8] 1995	Sugars, acids, vitamin C, amino acids	5 kg/cultivar, washed, mixed to pulp; for sugars and acids: 30 g pulp centrif.; clarified extract filtr. (0.45 μm)	Free sugars: HPLC, Waters–Millipore Sugar-Pack cartridge, 90°C, (RI) detection; succinic acid: HPLC, ORH1 Interaction cartridge, 45°C, UV detection; free ascorbic acid: HPLC, PLRP-100 A (250× 4.6 mm) UV detection; L-malic-, D-isocitric/citric acid enzymes	<1.45	Glucose, fructose, sucrose, sorbitol, raffinose, vitamin C, citric, L-malic, D-isocitric acids
Acids and sugars, simultaneously					
[9] 1996	Sugars, aliphatic acids	5 g/50% EtOH (blended)	GC–FID, TMS derivatives	–	Malic and citric acids, sucrose, glucose and fructose
[10] 1997	Sugars, sugar alcohols, acids,	5 g/10 ml 50% EtOH (blended, centrifuged, the supernatant diluted to 50 ml by 50% EtOH)	Capillary GC–FID, CP-Sil-5CB, DF 0.12, 25 m×0.25 mm I.D., TMS (HMDS+TMCS) and TMS-methoxime (methylhydroxylamine·HCl in pyridine+HMDS+TMCS) derivatives	r=0.919–0.999	Succinic, malic, citric, quinic, arach. acids, vitamin C, sorbitol, glucose, fructose, inositol, sucrose, trehalose, raffinose

[a] Notation: –=no data available; Met=methanol; EtOH=ethanol; EtAc=ethyl acetate; r=Pearson's correlation; FID=flame ionization detection; RI=refractive index; PDA=photodiode array.

Only three of ten publications [5,8,10] contain reproducibility data.

This paper describes an extension of our simultaneous acid/sugar quantitation procedure. Derivatization was performed in the presence of the matrix described earlier for different fruits, vegetables, honeys and mushrooms [11–26]. The various compounds were determined as their trimethylsilyl (TMS)-oxime ethers and/or esters, from one solution, by one injection. Recently, gas chromatography–mass spectrometry (GC–MS) was performed, evaluating sugar, sugar alcohol, acid and amino acid derivatives both on their total ion current (TIC) and selective fragment ion (SFI) values [15,17–23].

2. Experimental

2.1. Materials and reagents

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

Authentic apricot varieties Hungarian Kajszí (Apricot-1) and Bergeron (Apricot-2) were obtained from the Research Garden of the University of Horticulture and Food Industry (Péterimajor, Hungary). Peeled and seedless apricots were homogenized in a mortar and the pulps were used for derivatization (if not otherwise stated).

2.2. Preparation of fruit pulps prior to derivatization, according to the literature [5,7,8,10]

For alcoholic extractions 5 g pulp was weighed (with analytical precision) into a 100-ml Erlenmeyer flask and homogenized with 25 ml alcohol (50% or 80% ethanol or 80% methanol). After 15 min refluxing the supernatant was filtered through a glass filter paper (Whatman, Glass Microfibre Filter, GF/A, Cat. No: 1820912, 1.6 μm). The pulp was washed with the selected hot alcohol and the washes were collected with filtering until 50 ml stock solutions were obtained.

For centrifugation 5 g pulp was weighed (with analytical precision) into a tube and centrifuged (4000 rpm) for 15 min. The supernatant was filtered as above. The pulp was mixed and washed by 2×20 ml (centrifuged as above with each 20 ml water). Washes were filtered and collected until 50 ml stock solution was obtained.

2.3. Preparation of the TMS-oxime and TMS derivatives

Model solutions containing various amounts of major constituents ($0.5\cdot10^{-4}$–$5\cdot10^{-3}$ g malic acid, glucose, fructose and sucrose) and minor components ($5\cdot10^{-6}$–$2.5\cdot10^{-4}$ g hydroxymethylfurfural, amino acids, orthophosphoric acid, carboxylic acids, polyalcohols and sugars, shown all in Fig. 1A and B), as well as ~0.15–0.25 g apricot pulps or 2.00 ml alcoholic or aqueous stock solutions (containing approximately the corresponding amounts of acids and sugars) were evaporated to dryness in a rotary evaporator, at 50–60°C. The dehydrated residues were treated with 500 μl pyridine (containing 2.5 g hydroxylamine hydrochloride/100 ml) and were heated for 30 min at 70°C. The cooled samples were then trimethylsilylated with 1000 μl HMDS and 100 μl TFA in 2-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.

2.4. Separation of the TMS-oxime ether/ester derivatives

The apparatus was the Saturn II GC–MS system from Varian (Walnut Creek, CA, USA), supplied with an ion trap detection (ITD) system, a Varian 8200 autosampler and a septum-equipped programmable injector (SPI). The column used was from J&W Scientific (Folsom, CA, USA) (ID No. 2660081; DB-5, 30 m×0.248 mm; d_f=0.25 μm). The temperature program both of the column and that of the SPI, proved to be the ''reproducible optimum'', are shown in Table 2.

The temperature of the transfer line was 300°C. The actual parameters of the ITD system were defined by the automatic set-up mode.

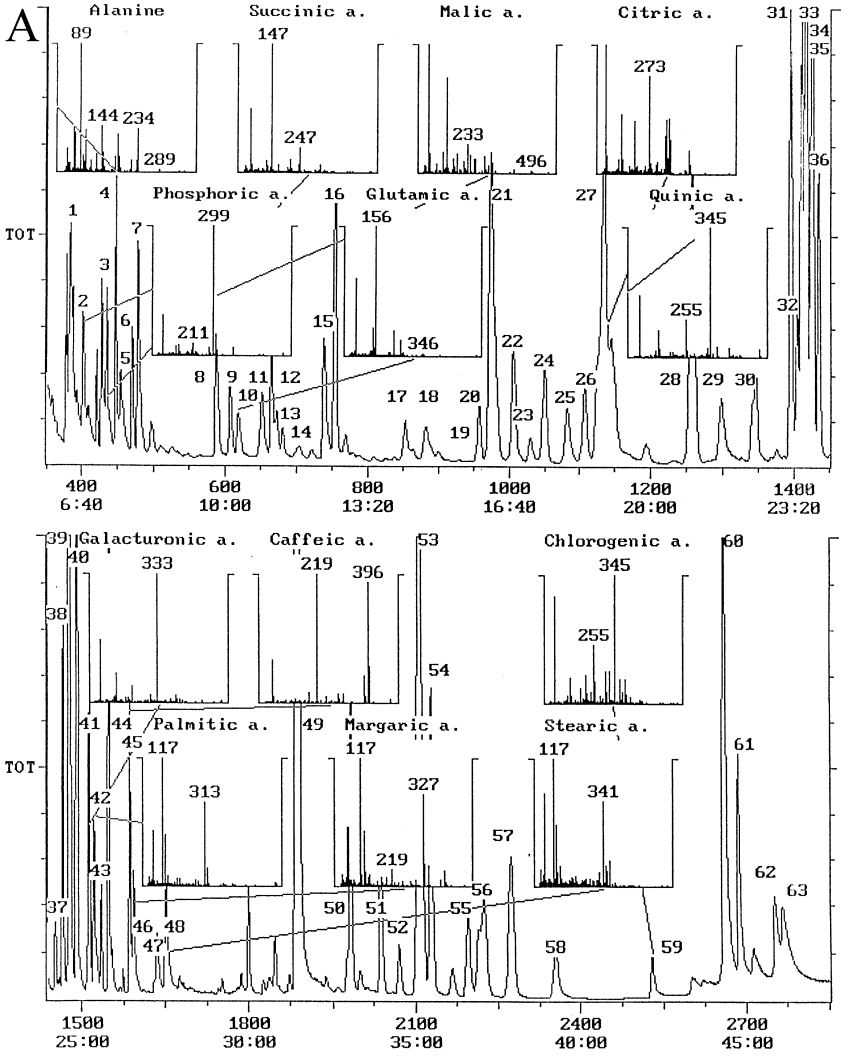

Fig. 1. GC–MS TIC chromatogram, containing also the spectra of selective fragment ions (SFIs) obtained from the TMS/TMS-oxime ether/ester derivatives of acids, sugars, sugar alcohols and amino acids from model solution (A), and from the A-1/1 apricot sample (B): 1=benzoic acid; 2=orthophosphoric acid (SFI=*m*/*z*=299); 3=succinic acid (SFI=*m*/*z*=147, 247, 335); 4=alanine (SFI=*m*/*z*=89, 130, 144); 5+7=levulinic acid 1,2; 6=serine; 8=malic acid (SFI=*m*/*z*=233, 335); 9=salicylic acid; 10=glutamic acid (SFI=*m*/*z*=156, 232); 11=cinnamic acid+hydroxymethylfurfurol; 12=3-hydroxybenzoic acid; 13=proline; 14=β-phenyllactic acid; 15=4-hydroxybenzoic acid; 16=tartaric acid; 17=3,5-dimethoxybenzoic acid; 18=veratric 3,4-dimethoxybenzoic acid; 19=γ-resorcylic 2,6-dihydroxybenzoic acid; 20=xylose 1; 22=xylose 2+arabinose 1,2+vanillic 3-methoxy-4-hydroxybenzoic acid; 22=gentisic 2,5-dihydroxybenzoic acid; 23+24=ribose 1,2; 25=*o*-coumaric 2-hydroxycinnamic acid; 26=β-resorcylic 2,4-dihydroxybenzoic acid; 27=shikimic+protocatechuic+4-methoxycinnamic acid+3,5-dihydroxybenzoic acid+citric acid+ fucose 1,2 (SFI=*m*/*z*=273, 347, 375, 465 for citric acid); 28=quinic acid (SFI=*m*/*z*=345); 29=*m*-coumaric 3-hydroxycinnamic acid; 30=syringic 3,5-dimethoxy-4-hydroxybenzoic acid; 31=mannitol; 32=4-hydroxycinnamic acid; 33=sorbitol; 34, 35=fructose 1,2; 36=3,4,5-trihydroxybenzoic acid; 37=caffeic acid 1 3,5-dihydroxycinnamic acid; 38=galactose 1; 39=galactose 2+glucose 1; 40=glucose 2; 41=galacturonic acid (SFI=*m*/*z*=333); 42=palmitic acid (SFI=*m*/*z*=117, 313); 43=glucuronic acid; 44=inositol+ferulic acid; 45=caffeic acid 2 (SFI=*m*/*z*=219, 396); 46=margaric acid (SFI=*m*/*z*=117, 327); 47=oleic acid; 48=stearic acid (SFI=*m*/*z*=117, 341); 49=sucrose; 50=trehalose; 51, 52=cellobiose 1,2; 53=turanose 1,2+maltose 1; 54=maltose 2; 55+57=palatinose 1,2; 56=gentiobiose 1; 58=gentiobiose 2+melibiose; 59=chlorogenic acid (SFI=*m*/*z*=255, 345); 59*=caffeoylquinic acid (SFI=*m*/*z*=255, 345, 396); 60=raffinose; 61=melezitose; 62+63=maltotriose 1,2. Detailed data in Tables 6 and 7.

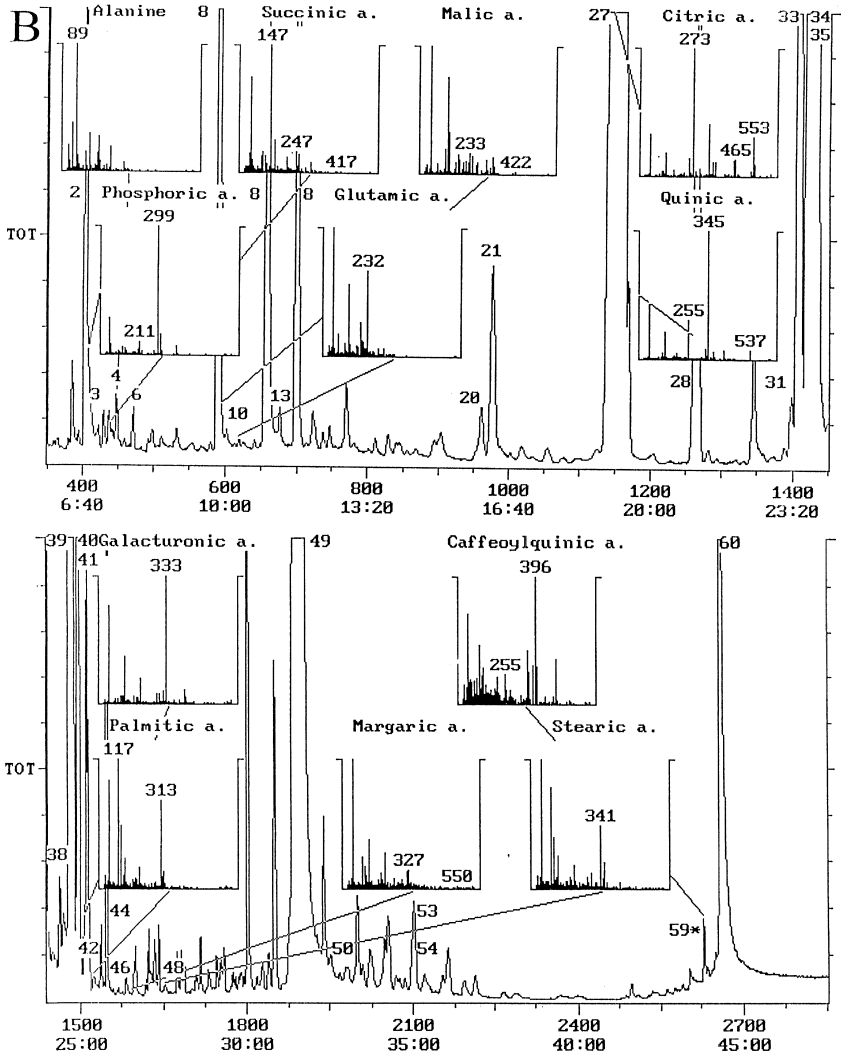

Fig. 1. (*continued*)

Table 2
Temperature program

Column				Injector			
Segment	Temperature	Rate	Time	Segment	Temperature	Rate	Time
1	60	0.0	2.0	1	60	0.0	2.00
2	120	20.0	3.0	2	320	180.0	1.44
3	155	6.0	5.83	3	320	0.0	6.00
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				

Table 3
Recovery of the sugar, acid, sugar alcohol and amino acid content of Apricot-1 sample in various extracts and without extraction, as their TMS derivatives, determined by GC–MS[a]

Derivatized in	Recovery (%) expressed in the percentages of the maximum yield														
	Acids				Sugars/sugar alcohols								Amino acids		
	Phosphor.	Malic	Citric	Quinic	Xylose	Mannitol	Sorbitol	Fructose	Glucose	Inositol	Sucrose	Raffinose	Alanine	Serine	Glutamic acid
Centrifuged[i]	20	34	17	25	18	21	31	26	25	32	37	–	34	–	–
Extr., 50% EtOH	84	100	90	92	88	88	97	84	85	97	95	52	75	58	100
Extr., 80% EtOH	80	92	84	84	88	84	92	72	77	89	93	60	90	76	100
Extr., 80% Met	94	100	94	96	94	89	100	84	85	100	100	80	98	80	100
Matrix, directly	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

[a] Notation as in Tables 3–7, as well as: [i]=liquor; Phosphor.=orthophosphoric acid; Met=methanol.

Actual automatic set-up conditions were as follows: mass range: 40–650 u; s/scan: 1.000; acquire time: 54 min; Fil/Mul delay: 420 s; peak threshold 0 count; mass defect: 100 mmu/100 u; background mass: 50 u.

3. Results and discussion

3.1. Study on the preparation of the fruit pulp

In order to consider the necessity of the extraction protocols described in the literature we compared three of them [7,8,10], to each other and to direct derivatization, (i.e., oximation/silylation is performed in the presence of the fruit matrix) (Table 3). Also in the case of apricot, similarly as described in details with other fruit matrices [15–17,19, 22,23,25,26], it has been repeatedly proven that direct derivatization is the method of choice: it obviates the use of internal standard(s) [10], that was declared also recently inevitable necessary, i.e., ''The use of two internal standards is advised as the first is useful for determining losses during the extraction and the second is to control the silylation procedure'' [10]. We performed external calibration derivatizing various amounts both of model solutions and of those of pulp matrix to be determined.

Comparing the efficiency of isolation proposals carried out as described in the literature, and compared to the direct derivatization method it turns out that (i) 80% methanol ensures the best recovery [5], in accordance with our earlier experience [24], (ii) 50% ethanol provides higher yield [9,10] than 80% ethanol [7], and, (iii) centrifugation is unsatisfactory for this purpose [8].

3.2. GC–MS studies in model solutions

GC–MS investigations performed with various expected apricot compounds of interest, in model solutions, including organic acids, orthophosphoric acid and amino acids, in the presence of enormous excess of saccharides of different degrees of polymerization (DPs), yielded promising results. Preliminary tests proved that all the acids, selected amino acids and sugars that could be assumed as minor constituents in apricots, including also those

Table 4
Reproducibility in the simultaneous quantitation of various amounts of fatty and carboxylic acids, amino acids and sugars: calculated from model solutions on the basis of their selective fragment ion (SFI) values[a]

Compounds (ng A)	Retention time (min)	SFIs (m/z)*	Arbitrary units/1 ng				RSD (%)
			A	B	C	Av.	
Phosphoric acid (4.18)	6.42	299	500	550	379	525	6.7
Succinic acid (4.39)	7.16	173, 247, 335	91	89	59	90	1.5
Alanine (40.60)	7.29	89, 130, 144,	–	–	45		
Serine (28.20)	7.51	204, 218, 306	–	–	59		
Glutamic acid (30.67)	10.12, 10.29	156, 232	–	98	97	98	≤1
Proline	11.22	142, 186, 216	–	–	44		
Asparagine	12.08	357	253	349	352		≤1
Lauric acid (2.73)	13.02	117, 129, 257	410	452	523	431	6.8
Shikimic acid (2.38)	18.58	255, 357, 372	161	216	239	227	7.1
Citric acid (4.25)	19.02	273, 347, 375	584	592	472	588	0.9
Fucose (0.55)	19.21, 20.07	277	25	31	41		
Miristic acid (4.02)	20.44	117, 129, 285	373	380	475	376	1.3
Galacturonic acid (0.95)	25.14, 25.35	332, 333	542	680	696	688	1.6
Palmitic acid (3.49)	25.23	117, 129, 313	388	372	454	380	2.9
Margaric acid (2.03)	26.34	117, 129, 327	345	342	311	332	5.6
Oleic acid (2.07)	27.16	117, 129, 339	71	93	97	95	2.9
Stearic acid (2.44)	27.32	117, 129, 341	502	413	361	387	9.5

[a] Notation: –=below the detection limit; *=fragment ions which proved to be satisfactory for quantitation [20–23]; B and C represent the corresponding amounts of A multiplied by 2 (B) and by 4 (C); underlined data have been omitted from the mean; Av.=averages of the mean obtained from A–C separate derivatization tests, injected at least three times each.

Table 5
Reproducibility in the simultaneous quantitation of various amounts of acids, sugars and sugar alcohols from model solution on the basis of TIC values[a]

Compounds (ng A)	Retention time (min)	Integrator units/1 ng (RSD, %, in parentheses)			
		A	B	C	Average
Malic acid (4.62)	9.50	163 (5.5)	194 (7.7)	195 (1.9)	195 (0.4)
Tartaric acid (5.04)	12.35	271 (4.8)	306 (5.2)	312 (2.8)	296 (7.5)
Xylose (2.12)	16.02, 16.17	501 (7.8)	471 (4.2)	–	486 (4.4)
Quinic acid (2.23)	21.04	371 (3.5)	380 (4.3)	376 (2.8)	376 (1.2)
Mannitol (0.94)	23.18	541 (1.6)	524 (4.5)	540 (2.7)	535 (1.8)
Sorbitol (4.49)	23.33	567 (2.6)	569 (6.8)	580 (3.5)	572 (1.2)
Fructose (13.88)	23.39, 23.48	950 (1.5)	883 (4.9)	960 (1.1)	931 (4.5)
Galactose (0.78)	24.28, 24.40	547 (4.8)	606 5.8)	–	576 (17)
Glucose (25.00)	24.40, 24.52	592 (4.3)	556 (5.9)	579 (2.4)	576 (3.2)
Inositol (1.08)	25.48	561 (5.6)	618 (4.8)	623 (2.1)	600 (5.7)
Sucrose (120.5)	31.28	337 (2.9)	344 (7.2)	336 (0.5)	339 (1.3)
Trehalose (0.94)	33.05	360 (2.9)	374 (7.8)	368 (2.8)	367 (1.9)
Cellobiose (0.88)	34.00, 34.32	226 (1.2)	193 (12)	204	208 (8.1)
Turanose (0.98)	35.05, 35.21	280 (11)	286 (9.3)	–	283 (1.5)
Maltose (3.90)	35.09, 35.33	275 (1.0)	238 (12)	–	250 (8.5)
Raffinose (4.30)	44.19	651 (3.4)	660 (13)	701 (7.1)	670 (4.0)
Melezitose (0.91)	44.47	252 (7.3)	244 (11)	273 (15.0)	256 (5.8)

[a] Notation as in Table 4; –=no data available; B and C represent the corresponding amounts of A multiplied by 2 (B) and by 4 (C).

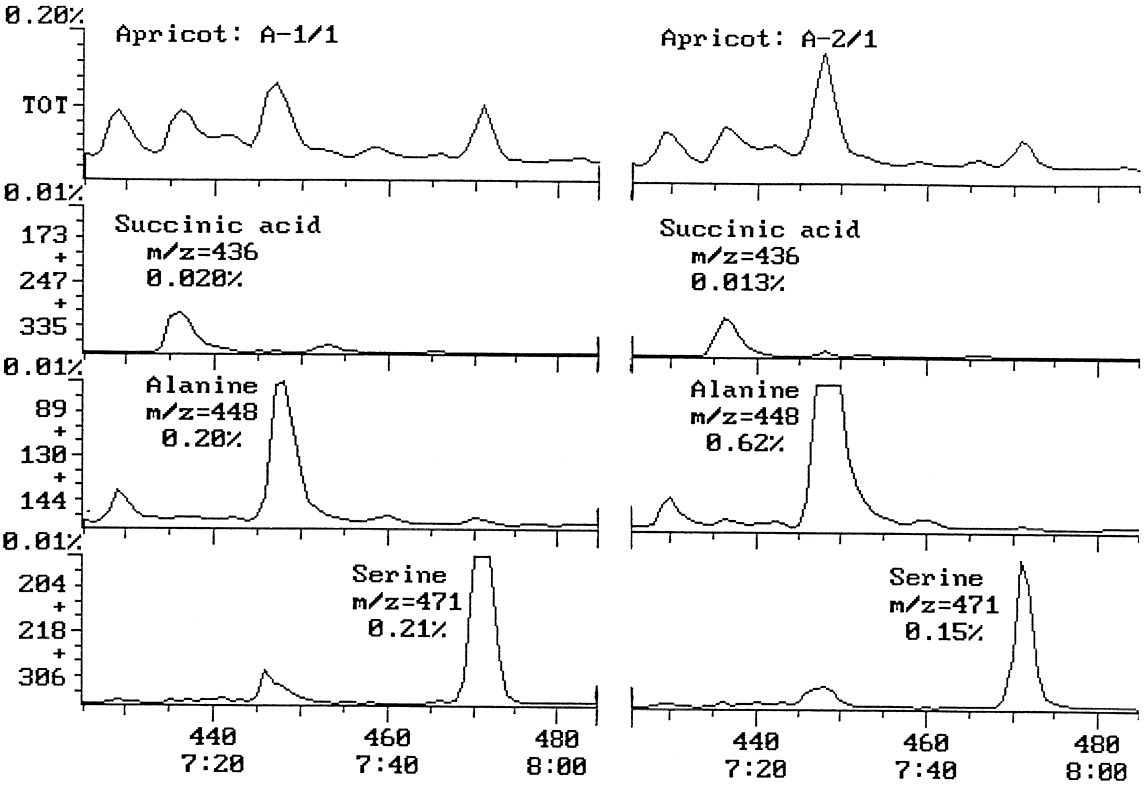

Fig. 2. Selected parts of the GC–MS TIC chromatogram, between 405–485 scan, containing the SFIs in separate display lines for succinic acid, alanine and serine determined in A-1/1 and A-2/1 apricot samples. Detailed data in Table 6 and in the text (Section 3.2).

which could not be resolved completely from their neighbors, or those which are covered with unknown impurities, or simply are present in extremely low concentrations can be evaluated on the basis of their SFI values (Fig. 1A and B, 63 compounds, selected of them illustrated also by their SFIs). Introductory investigations carried out with two apricot samples (Apricot-1, Apricot-2) provided information concerning the type and the quantity of minor compounds to be identified and measured in our matrices. With this knowledge, the trace and main constituents were measured in model solutions (Tables 4 and 5). Minor constituents in the low ng concentration level, in the presence of each other were determined mainly on the basis of their SFI values [20–23] (Table 4).

As an example, the evaluation possibility of the succinic acid–alanine–serine mixture can be seen in a spectacular manner, based on their SFI values, in Fig. 2. The very small signals of succinic acid, alanine and serine are covered by unknown impurities, indicated by the total of ions (TOT – given by the software of the apparatus is identical with the commonly used TIC values – Fig. 2, first display

Table 6
Reproducibility in the simultaneous quantitation of amino, fatty and carboxylic acids and sugars from apricot samples determined on the basis of their selective fragment ion (SFI) values[a]

Constituents	Retention time (min)	SFI(s) (*m*/*z*)	Constituents (%) based on the dry matter contents of samples*								
			A-1/1	A-1/1B	A-1/1C	A-1/1D	A-1/2	A-1/3	A-2/1	A-2/2	A-2/3
Phosphoric acid	6.42	299	0.58	0.59	0.59	0.62	0.64	0.68	0.42	0.71	0.45
			(1.8)	(2.8)	(0.9)	(1.2)	(1.1)	(0.20)	(1.5)	(1.9)	(3.0)
Succinic acid	7.18	173, 247, 335	0.020	0.042	0.045	0.049	0.022	0.017	0.013	0.029	0.017
			(14)				(16)		(16)	(24)	(20)
Alanine	7.29	89, 130, 144,	0.20	0.17	0.19	0.15	0.36	0.42 (14)	0.62	0.19	0.72
			(2.6)	(6.6)	(2.3)	(6.3)	(14)		(0.10)	(4.6)	(1.8)
Serine	7.51	204, 218, 306	0.21	0.21	0.26	0.36	0.25	0.32	0.15	0.19	0.30
			(1.6)	(8.3)	(13)	(6.1)	(3.1)	(10)	(0.1)	(0.1)	(3.4)
Glutamic acid	10.20	156, 232	0.27	0.54	0.56	1.03	0.29	0.42	0.021	0.033	0.071
			(5.1)	(4.8)	(4.1)	(5.8)	(13)	(6.1)	(7.0)	(5.7)	(2.2)
Proline	11.22	142, 186, 216	0.25	0.21	0.18	0.27	0.28	1.05	–	–	0.045
				(11)	(14)	(13)					
Citric acid	19.02	273, 347, 375	7.20	8.64	7.56	8.47	7.45	6.38	4.84	6.10	3.02
			(3.7)	(3.2)	(3.1)	(1.4)	(2.3)	(0.6)	(1.1)	(2.6)	(1.8)
Fucose	19.21, 20.07	277	0.011	0.013	0.011	0.012	0.086	0.072	0.13	0.10 (2.8)	0.071
			(12)	(15)	(18)	(2.6)	(0.30)	(1.5)	(2.1)		(1.8)
Galacturonic acid	25.14, 25.35	332, 333	0.013	0.034	0.033	0.075	0.014	0.018	0.012	0.013	0.020
			(3.1)	(1.7)	(1.4)	(2.6)	(2.8)	(3.6)	(7.2)	(5.1)	(2.2)
Palmitic acid	25.23	117, 129, 313	0.021	–	–	–	0.020	0.022	0.014	0.038	0.019
			(10.5)				(11)	(0.10)	(1.7)	(5.5)	(1.8)
Stearic acid	27.32	117, 129, 341	0.016	–	–	–	0.012	0.008	0.009	0.015	0.008
			(2.7)				(12)	(11)	(1.6)	(11)	(6.9)
Turanose	35.09	361	0.025	–	–	–	0.037	0.022	0.030	0.045	0.010
			(6.4)				(1.3)	(6.6)	(8.8)	(2.3)	(4.3)
Maltose	35.09, 35.21	361	0.014	–	–	–	0.012	0.019	0.014	0.024	0.028
			(2.9)				(3.4)	(0.20)	(4.4)	(0.50)	(0.50)
Caffeoylquinic acid (59**)	43.50	396	0.18	–	–	–	0.15	0.12	0.21 (1.3)	0.24 (4.0)	0.12
							(4.1)	(6.0)			(5.9)

[a] Apricot-1 (A-1/1–A-1/3) and Apricot-2 (A2/1–A-2/3) samples were obtained after various harvesting times: at 14/07/98 (A-1/1), at 16/07/98; (A-1/2 and A-2/1) at 21/07/98; (A-1/3 and A-2/2), and at 24/07/98 (A-2/3). A-1/1B–A-1/1D are stored versions of sample A-1/1, stored at +2°C, 85% relative humidity, until 21/07/98 (A-1/1B), 27/07/98 (A-1/1C) and 03/08/98 (A-1/1D), respectively; (*=the dry matter content of samples varied between 13.1 and 17.9% (w/w); –=no data available; Note: RSD data, below percentages in parentheses, have been calculated from two or three separate derivatizations of apricot pulps, eluting three times each; selected constituents were evaluated later on the basis on a single, saved chromatogram: in these cases RSD data fail; 59**=4-caffeoylquinic or 3-caffeoylquinic acid, i.e., crypto- or neochlorogenic acid (Fig. 1B).

Table 7
Reproducibility in the simultaneous quantitation of acids, sugars and sugar alcohols from apricots determined on the basis of total ion current (TIC) values[a]

Compounds	Retention time (min)	%, Calculated on the basis of the dry matter contents of samples* (RSD, %, in parentheses)								
		A-1/1	A-1/1B	A-1/1C	A-1/1D	A1/2	A1/3	A2/1	A2/2	A2/3
Malic acid	9.50	7.70 (4.8)	6.61 (1.8)	5.88 (0.7)	5.24 (0.9)	6.03 (2.3)	7.03 (2.7)	15.0 (4.2)	14.6 (1.7)	13.5 (2.6)
Xylose	16.02, 16.17	0.072 (1.4)	0.21 (3.2)	0.17 (2.9)	0.21 (1.2)	0.10 (2.7)	0.061 (2.9)	0.11 (4.6)	0.073 (4.3)	0.083 (2.9)
Quinic acid	21.04	0.32 (1.4)	0.35 (1.3)	0.35 (0.9)	0.53 (1.6)	0.52 (3.9)	0.34 (2.9)	1.08 (2.0)	0.59 (5.0)	0.54 (1.4)
Mannitol	23.18	0.0077 (8.5)	0.0080 (0.8)	0.0096 (5.2)	0.011 (8.6)	0.0063 (7.2)	0.0043 (4.5)	0.0049 (3.9)	0.0029 (10)	0.0044 (11)
Sorbitol	23.33	0.92 (0.9)	0.79 (1.9)	0.51 (1.6)	0.63 (0.5)	1.63 (3.0)	0.82 (2.2)	1.62 (1.1)	3.08 (1.9)	2.99 (0.9)
Fructose	23.39, 23.48	5.19 (1.6)	8.01 (3.3)	9.01 (2.7)	11.7 (1.0)	5.89 (2.8)	3.14 (3.0)	4.73 (1.5)	5.86 (3.8)	3.73 (2.4)
Galactose*1	24.32, 24.44	0.027 (1.6)	0.0080 (16)	0.0084 (5.4)	0.0081 (7.3)	0.0080	0.0081	0.012 (11)	0.011 (6.4)	0.012
Glucose	24.40, 24.52	11.2 (3.5)	14.6 (2.8)	15.3 (0.9)	18.2 (1.8)	13.0 (1.8)	9.91 (3.5)	10.7 (2.9)	18.4 (1.3)	13.1 (1.5)
Inositol	25.48	0.44 (2.1)	0.31 (4.6)	0.28 (2.5)	0.29 (2.0)	0.39 (6.6)	0.35 (1.6)	0.33 (2.2)	0.30 (3.8)	0.28 (1.9)
Sucrose	31.28	43.5 (3.5)	36.7 (2.7)	33.5 (2.1)	25.4 (2.8)	40.9 (3.1)	47.7 (3.1)	40.8 (1.9)	38.7 (2.2)	41.4 (1.0)
Trehalose	33.09	0.0067 (12)	0.0077 (16)	0.0084 (8.6)	0.0073 (9.8)	0.0066 (1.1)	0.0065	0.0073 (19)	0.011 (19)	0.0096 (5.9)
Disaccharides[t]	~29–~36	0.61	0.51 (4.5)	057 (1.8)	0.61 (3.0)	0.65	0.73	0.72	0.75	0.89
Raffinose	44.19	0.96 (6.9)	1.61 (4.9)	1.95 (0.1)	3.21 (1.2)	0.64 (9.2)	0.47 (7.6)	1.30 (3.0)	0.74 (2.1)	0.73 (2.3)

[a] Notation as in Tables 3–6, as well as/or: *1=calculated on the basis of its resolved oxime (retention time: 24.32 min [18,19]); disaccharides[t]=the total of constituents eluting in the region of ~29–~36 min, providing the characteristic fragment m/z=361.

line). As is evident, these TOT=TIC values are different for both samples: they are relatively large values and do not at all reflect the presence of the three constituents. These three compounds have been determined by their SFIs: partly on the basis of our recent experiences covering the fragmentation patterns and quantitation possibilities of members of various homologous series of acids [20–23], partly on this study obtained with the TMS amino acids, including alanine and serine. The SFI for succinic acid (Fig. 2, second display line, at 436 scans), alanine (Fig. 2, third display line, at 448 scans) and serine (Fig. 2, fourth display line, at 471 scans) allowed their quantitation (Table 6) on the basis of those fragments shown on the vertical axes of SIM display lines (succinic acid: m/z=173=[M−TMSO]$^+$, m/z=247=[M−CH_3]$^+$, m/z=335=[M+TMS]$^+$; alanine: m/z=89=[M−2TMS]$^+$, m/z=130=[H–CH_3–Si$(CH_3)_4$]$^+$, m/z=144=[M−TMSO]$^+$; serine: m/z=204=[M−TMSCOO]$^+$, m/z=218=[M−TMSCO]$^+$, m/z=306=[M−CH_3]$^+$).

The quantitation of the main constituents, on the basis of their TIC values was summarized in Table 5. The reproducibility of all of these data (Tables 4 and 5) proved to be acceptable (average RSD=3.8%).

3.3. Quantitation of the constituents of apricots as a function of their harvesting time and storage conditions

The amounts of the various minor and major constituents of apricot samples are presented in Tables 6 and 7, and in Figs. 1B and 2. Minor compounds in apricots were quantitated mainly on

Table 8
Constituents of apricots: identified and measured from one solution by one injection as their TMS-oxime ester/ether derivatives[a]

Constituents	Constituents, in total by groups (%) calculated on the dry matter basis of samples								
	A-1/1	A-1/1B	A-1/1C	A-1/1D	A-1/2	A-1/3	A-2/1	A-2/2	A-2/3
Sugars	61.7	61.7	60.6	59.4	61.4	62.2	58.6	64.7	60.1
Sugar alcohols	1.36	1.11	0.80	0.93	2.02	1.17	1.95	3.38	3.27
Acids	16.0	16.2	14.4	14.9	14.9	14.6	21.6	22.3	17.7
Amino acids	0.79	1.13	1.19	1.81	1.14	2.21	0.79	1.48	1.18
In total	79.9	80.1	77.0	77.0	79.5	80.2	82.9	91.9	82.3

[a] Notation as in Tables 3–7.

the basis of their SFI values (Table 6, [20–23]) with an average reproducibility of 4.3% RSD. The main constituents, determined by their TIC values provided an average reproducibility of 2.4% RSD (for components present in concentrations of ≥1%) and 6.3% RSD (for components present in concentrations of ≤1%). The evaluation of the TMS sugar oximes was based on the fact that the ratios of the *syn* and *anti* forms are stable, independent of their amounts analyzed and characteristic only to the sugar species to be measured [18,19].

As to the composition of the two apricot cultivars, considerable differences have not been found, with the only exception of their malic and citric acid contents.

Regarding the impact of the successive harvesting dates, significant changes in compositions could not be determined either.

Evaluating the amounts of constituents measured in the A-1/1 apricot under the 20-day storage period (A-1/1B, A-1/1C, A-1/1D samples), it can be stated that (i) the acid contents of stored apricots did not vary, (ii) however, the simultaneously decreasing amounts of sucrose and increasing amounts of glucose and fructose reveal the hydrolysis of sucrose (Table 7, in order of increasing storage times, expressed in %: sucrose, 43.5, 36.7, 33.5, 25.4; glucose, 11.3, 14.6, 15.3, 18.2; fructose, 5.19, 8.01, 9.01, 11.7), while (iv) the increasing amounts of raffinose (Table 7: 0.96, 1.61, 1.95 and 3.21%) can be attributed to the hydrolysis of saccharides of higher DPs.

The efficiency of the proposed method is shown by the total of the identified and quantitated compounds (Table 8) that proved to be close to 80%, from one solution by a single injection, in the concentration range of five-orders of magnitude.

4. Conclusion

In summary, it can be stated that the extension of our ''simultaneous sugar/acid quantitation'' method resulted in the reliable and reproducible determination of the constituents of apricots, including (i) aliphatic and aromatic carboxylic acids, (ii) mono-, di- and trisaccharides and sugar alcohols, as well as, (iii) amino acids (alanine, serine, glutamic acid, proline) and (iv) in the presence of the fruit matrix, without any pretreatment and without the use of internal standards, (needed earlier to correct the loss under extraction and derivatization), in the concentration range of 10^{-3}–≥40%, as their TMS-oxime ether/ester derivatives, by GC–MS.

Quantitation has been performed both on the basis of TIC and on those of the SFI values resulted in identification and quantitation of ~80%, of the fruit matrix (related to their dry matter contents).

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